1982

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Iowa State University PH.D. 1982

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Role of soybean in plant-particle pneumonia of swine

by

Henry Grady Wall

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1982

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ABSTRACT

Objectives of this investigation were a) to characterize a naturally occurring plant-particle pneumonia in swine and b) to reproduce the disease in experimental pigs using soybean particle suspensions.

The incidence and lung histopathology of natural plant-particle pneumonia were determined by examination of lung tissues from 62 confinement-reared, self-fed pigs; 26 confinement-reared, floor-fed pigs; and 444 pig necropsies accessioned during a 5 year period.

Characteristics of experimental soybean-induced plant-particle pneumonia were determined by clinical observations and gross, histological, ultrastructural and microbiological examinations of the respiratory tract. Ninety-three pigs given soybean particles transtracheally and 36 control pigs were studied.

In the naturally occurring disease, plant particles were associated with granulomatous lung lesions in 25 of 62 self-fed pigs. Particles morphologically consistent with soybean were found in 7 of the 25 pigs. Six of 9 pigs among 444 pigs examined retrospectively had soybean particles in granulomatous lung lesions. Microscopically, lesions in affected pigs from both groups consisted of neutrophils admixed with macrophages and multinucleated giant cells in alveolar and bronchiolar exudate. Plant particles 20 to 100 μm were free in alveoli, in phagocytes and in granulomas.

Experimentally induced plant-particle pneumonia was characterized clinically by coughing and dyspnea. At necropsy, there was gross bullous emphysema, lung hemorrhage, and yellow to gray lung mottling. Microscopically, there was a sequential transition from acute bronchopneumonia to granulomatous pneumonia. Ultrastructurally, there were interstitial foreign body granulomas which formed when multinucleated giant cells became entrapped by connective tissue after alveolar epithelium was destroyed. When pigs were given multiple doses of soybean lesions were simultaneously suppurative and granulomatous, similar to those seen in the natural disease.

GENERAL INTRODUCTION

The American Veterinary Medical Association Council on Research has determined that respiratory tract disease is a major swine health problem requiring investigation. The annual cost of pneumonia-associated swine production losses in the United States has been estimated at $250 million. That estimate exceeds loss estimates for diarrhea, atrophic rhinitis, arthritis, reproductive failure, swine dysentery, internal parasitism and external parasitism (63). Respiratory tract disease causes swine death losses and reduced feed efficiency (63,81). The present trend of intensive swine production in totally enclosed facilities has revealed swine health problems that require a better understanding of the influence of environmental factors on swine (3,5,28,80). Microbes, toxic gases, air temperature, relative humidity and ventilation are environmental factors known to significantly influence swine health (5,29,65,132,134).

There are differing views on the effect of dusts on swine health. Curtis et al. concluded that even though dust concentration in poorly ventilated swine facilities can exceed the threshold limit value (10 mg/m^3) for human industrial occupancy, the direct influence of airborne dust on performance of a healthy pig is minimal (30). They have further reported that swine house dusts are probably inert and do not cause formation of fibrous pulmonary lesions when deposited in the lung (30,31). Measurements of the concentration and size of dust particles in swine-house air revealed that 90-95% of the particles were 5 μm or less in diameter (14,65);

consequently, the particles are likely to be inhaled into the respiratory lobules (1,50).

Plant-particle associated swine pneumonias have been described. Plant particles recognized in the lung are commonly over 5 μm in diameter. Plant-particle associated pneumonias in swine are due to inhalation of plant particles derived from feed or bedding materials (27,57,147). Only a few reports have identified effects on the swine lung attributable to specific plant materials (35,57,58).

Soybean meal is a major source of protein in swine feed in the United States. An etiologic role for soybean meal in plant-particle associated pneumonia in swine has not been established. The histologic demonstration of soybean particles in the lungs of swine from an Iowa commercial swine farm provided the impetus for this investigation on the role of soybean meal (SBM) in development of pneumonia in swine.

The objectives of this research were: (1) to determine the incidence and character of lung lesions in swine naturally exposed to feed particles that included soybean meal; (2) to characterize effects of transtracheally inoculated soybean particles on the clinical condition and respiratory tract structure of swine; and (3) to compare natural plant-particle associated pneumonia in swine with experimental soybean meal-induced pneumonia in swine.

Principal investigative methods used in these studies were clinical observation and gross, histological, ultrastructural, and microbiological examinations that included bacterial isolations and complement-fixation tests for mycoplasmas.

This dissertation is presented in alternate format including four manuscripts to be submitted to Veterinary Pathology. All manuscripts are presented in the format required by Veterinary Pathology. References cited in each manuscript are included with that manuscript and conform to journal format. The manuscripts are preceded by a general introduction and literature review. General discussion and conclusions, literature cited, and acknowledgements follow the manuscripts. Literature cited refers to citations in the general introduction and general discussion and is presented in journal format.

The Ph.D. candidate, Henry G. Wall, was the principal investigator for each of the studies and is the senior author of each manuscript.

LITERATURE REVIEW

Effects of Noninfectious Environmental Pollutants on Swine

Introduction

The present trend of raising large swine herds in completely enclosed facilities achieves more efficient utilization of land, equipment and labor. This swine production trend has created increased concern about the direct effects of noninfectious environmental pollutants on swine health and their effects on infectious disease in swine (3,5,28,80). Toxic gases, odorous vapors and dusts are the principal noninfectious pollutants in enclosed swine facilities (5,14,29,32,48,65,71,74,76,114,132).

Effects of toxic gases and odorous vapors

Carbon dioxide and methane are asphyxiants but are usually not present at toxic levels in swine confinement facilities. Pigs are tolerant to confinement unit levels of hydrogen sulfide that do not exceed 10 ppm. Hydrogen sulfide levels exceeding 400 ppm are lethal to pigs. When subfloor pit stored waste is agitated, lethal hydrogen sulfide concentrations that exceed 1000 ppm are known to occur (29,132).

Ammonia is the most frequently encountered toxic gas in swine confinement units. Growth and food intake of healthy pigs may be reduced 10% or more when aerial ammonia exceeds 50 ppm. Bacterial clearance from the respiratory tract is also impaired. Ammonia irritates swine mucous

membranes, however, there are no consistent gross or histopathologic lesions (29,37).

Carbon monoxide originates from incompletely burned fuel. Toxic levels can build up when fuel-burning sources are used to provide supplemental heat in inadequately ventilated farrowing houses. This gas causes either abortion of full term feti or weak pigs that usually die shortly after birth. In cases of carbon monoxide poisoning, fetal and maternal carboxyhemoglobin levels are excessive (20,21).

No pathologic effects in swine are directly associated with odorous vapors. Vapors are adsorbed to dust particles (32,48).

Effects of dust on swine health

Several authors agree that feed, bedding materials and solid excreta are principal sources of dusts in enclosed swine facilities (14,31,65). However, there are differing views on the pathogenicity of particulates for the pig respiratory tract. The information that follows summarizes previous findings pertinent to swine health effects associated with either natural or experimental exposure of pigs to environmental dust. Effects of particulates on the respiratory tract are emphasized.

In 1936, Innes proposed that dust inhalation was not responsible for coughing and pneumonia in pigs (55). Shanks tested Innes' theory. Pigs bedded on meal mill dust reached market weight faster than pigs bedded on straw. Only one pig that was bedded on dust developed pneumonia and that result was equivocal (122).

Swine pneumonia associated with aspirated vegetable material was described by Whittlestone. He encountered 57 histologically similar pneumonias in 323 pigs with pneumonic anterior lung lobes. Forty of the 57 histologically similar pneumonias had fragments or remnants of vegetable material in microscopic lung lesions. Vegetable material were found in bronchioles and alveoli and multinucleated giant cells usually surrounded the material. Most lesions were diffuse, but encapsulated foci were occasionally observed. Alveolar collapse was evident in nearly all cases. The inflammatory reaction was judged to be chronic in most cases; however, acute pneumonia attributed to secondary bacterial invasion occurred in some pigs. Clinical signs were mild in uncomplicated cases of vegetable particle associated pneumonia (147).

Corner and Jericho reported that foreign material derived from cereal feed was found in 40 of 68 lungs from test and control pigs used in a mycoplasmosis experiment. Pigs with foreign material in their lungs had been housed in an enclosed piggery in which feed was dispensed from buckets into floor-based troughs. Pneumonia, characterized by granulomas, giant cells and numerous intrabronchiolar and intraalveolar macrophages, was observed in lungs of pigs that inhaled feed particles. Six more pigs had granulomatous pneumonia without recognizable plant material. Plant particles in bronchioles evoked a neutrophilic infiltrate. Bronchiolar epithelial erosion occurred at points of contact with large particles. Bronchiolar epithelium adjacent to inhaled particles was also hyperplastic. Particles up to 300 μm in diameter were in bronchioles and

particles up to 50 μm in diameter were in the alveolar ducts and sacs. Pulmonary lymphoreticular hyperplasia was also observed; however, no relationship between presence of plant particles and lymphoreticular hyperplasia was established (27). Lymphoreticular hyperplasia is commonly seen in Mycoplasma hyopneumoniae infections (70,110).

Jericho and Harries described swine pneumonia associated with a dusty mixture of peas, wheat and barley. Pigs introduced into a feeder barn at about 6 weeks of age were transferred to progressively higher numbered pens (1 to 16) until they reached market weight. Pigs in pens 1 through 4 obtained their feed from buckets. Respiratory disease was primarily present in pigs in pens 3 through 8. Only a few pigs in higher numbered pens were affected. Clinical signs included labored breathing, depression and weight loss. Eight pigs were found dead and 15 clinically ill. Four ill pigs were killed and necropsied. Each of the 4 pigs had acute alveolitis. Bronchiolar epithelial squamous metaplasia was observed. One pig had granulomatous pneumonia. Feed particles, commonly measuring 15 μm wide, were found in lymphatics, air passages, alveoli and regional lymph nodes. When feed was treated with fish oil and ventilation changed to minimize dusty conditions, the incidence of respiratory disease abruptly decreased (58).

Jericho compared the effects of dry feed and wet feed on lungs of vitamin A deficient pigs from the same herd described by Jericho and Harries (58) and vitamin A fortified pigs from another herd. Pigs deficient in vitamin A receiving dry feed had more particles in their

lungs than either of the other 2 groups. Starch particles found in lungs of experimentally treated pigs were usually not associated with accumulations of macrophages or giant cells and lymphoid proliferation was also absent. Jericho concluded that starch particles were of little significance in pneumonic processes (57).

Nakamura compared the histologic structure of lungs from pigs fed a powdered ration, pigs fed a mixture of powdered ration and regular ration, and pigs that had retarded growth. Lesions of aspiration pneumonia with starch granules were most numerous in pigs with retarded growth. There was no positive tendency for powdered rations to induce aspiration pneumonia (82).

Dust, viable microbes and contaminant gas content of 4 fattening units and 2 farrowing houses were determined by Kovacs et al. Dust and viable microbe content was generally highest in winter. When dry feed was dispensed from automatic feeders, 110 to 430 dust particles and 550 to 1600 viable microbes were found in one ml of air. The diameter of 90-95% of the dust particles was 0.5 to 2.0 μm while 5-10% of the particles had diameters from 2 to 10 μm. Ammonia was present at levels up to 18 ppm in atmospheric air. Dusty fattening pens accounted for most (87%) pneumonia cases; whereas, most severe pneumonias occurred in pigs from less dusty heated pens with high ammonia levels. These researchers proposed that organic antigens derived from mixed feed dust may provide an allergic basis for development of respiratory disease in pigs in enclosed facilities (65).

Doig and Willoughby exposed one week-old pigs in environmental chambers to 3 μm to 5 μm diameter aerosolized cornstarch particles at 6 mg/cu ft of air for 6 weeks. There was no effect on respiratory tract structure, mean daily gain, or frequency of coughing (35).

Curtis et al. exposed pigs in air pollutant chambers to continuous aerial levels of ammonia, hydrogen sulfide and swine-house dust at levels equal to or greater than those normally encountered in swine houses. Approximately 50% of the dust particles were 5 μm or less in diameter. Pollutants were administered alone or in combinations for 17 to 109 days. Rate of gain and respiratory tract structure of healthy pigs were not directly affected by ammonia, hydrogen sulfide and dust at levels and combinations commonly encountered in commercial swine confinement facilities (30).

The preceding literature review regarding pathologic effects of particulates on the pig respiratory tract reveals that differences in particle sizes may account for the character and severity of observed lesions. Except for the increased incidence of pneumonia reported by Kovacs et al. (65), particles less than 5.0 μm in diameter had no effect on the respiratory tract structure of pigs (30,35,57). Pulmonary structural alterations were most severe in pig lungs examined by Whittlestone (147), Corner and Jericho (27) and Jericho and Harries (58). These authors observed particles ranging in size from 15 μm to 300 μm in diameter in swine lung lesions.

Although most of the reports concerning effects of particulates on swine health do not emphasize the role of feeding practice, there is an indication that trough-fed dry rations and self-fed rations may be predisposing factors to plant-particle aspiration pneumonia. Bundy and Hazen determined dust levels and pig activity levels in swine confinement systems associated with different feeding methods. Animal activity and dust levels were higher when pigs were self-fed than when pigs were floor-fed twice daily. Floor-fed pellets caused less dustiness than floor-fed ground feed. No significant difference was found between dust levels for pellets and ground feed in self feeders. There was no significant difference in dust concentration when dry ground feed was fed twice daily or wet feed was fed twice daily in a trough on stainless steel slatted floors. A 50% reduction in dust level was achieved by 35 cfm air ventilation. These researchers also reported that 95% of the particles in the air of swine confinement facilities were less than 5 μm in diameter (14).

Lindqvist compared the incidence of pneumonia in floor-fed fattening pigs with trough-fed fattening pigs given pelleted or dry meal rations (69). In 21 of 32 comparisons, a significantly lower incidence of pneumonia was found in floor-fed pigs. Like Bundy and Hazen (14), Lindqvist also concluded that trough-feeding resulted in more dust than floor-feedings.

Factors that Determine if a Specific Dust Causes Disease

Particle concentration, size, shape, and chemical nature; host respiratory tract anatomy, breathing pattern and duration of exposure

are factors that determine if a specific dust causes disease (12,168). When atmospheric air is not excessively dusty, mucosal ciliary action clears 60% of particles inhaled by healthy humans. Thirty per cent of inhaled particles are phagocytized by alveolar macrophages. The remaining 10% of the inhaled particles are removed by lymphatics (108). Particles less than 1 μm in diameter and most particles between 1 to 5 μm in diameter can be deposited in alveoli (1,50,108). Particles under 0.02 μm in diameter can penetrate the alveolus (108).

Mechanisms that influence the deposition of particles in the respiratory tract are impaction, sedimentation, Brownian motion, turbulent diffusion, and to a lesser extent electrostatic forces. The foregoing mechanisms also interact with particle size and shape, respiratory tract anatomy, and breathing pattern to influence deposition pattern of inhaled particles (83).

Impaction accounts for the efficient nasal filtration of inhaled particles over 10 μm in diameter (83,131). Turbinates of a pig cause inhaled air currents to assume a swirling pattern in the nasal cavity; consequently most particles over 5 μm impact upon the nasal mucociliary blanket (131). Particles on the mucociliary blanket are swept to the pharynx and swallowed or expelled by sneezing. The majority of particles over 10 μm that are not cleared by nasal filtration impact on the posterior pharyngeal mucosa and are swallowed or coughed out. The remaining particles over 10 μm impact upon the tracheal mucosa or mucosa of early bronchial division and are eventually cleared by mucociliary transport (83,131).

Inhaled particles less than 5.0 μm in diameter and down to 0.2 μm

tend to remain suspended in flowing air. However, when air reaches the lung periphery, flow rates are reduced so low that gravitational forces cause particles to settle against the wall of bronchioles and alveoli. These events are descriptive of sedimentation, which is probably the most important mechanism for deposition of inhaled particles in the peripheral lung lobules. Brownian motion is the main mechanism accounting for deposition of particles 0.1 μm or smaller in the respiratory tract. Until deposited, these particles are in continuous random motion as a result of their bombardment by gas molecules (83).

Breathing pattern and airway structure influence turbulence of inhaled air. Deep breathing increases inspiratory velocity and promotes deposition of particles in peripheral lung lobules. Highly branched airways cause more particles to impact and sediment against the epithelium lining of the more proximal branches of the respiratory tract (12).

Effective aerodynamic diameter and hygroscopic property of a particle also influence its deposition. Effective aerodynamic diameter of a particle is determined by its size, shape, and density. Particles that can absorb or liberate water can vary in density. Asbestos particles, up to 300 μm long, orient parallel to the airstream and behave aerodynamically like 1 μm diameter spherical particles (12).

Particles less than 0.02 μm in diameter that are absorbed are readily cleared by lymphatics (67,129). Most alveolar deposited particles that cannot penetrate the epithelium are phagocytized by alveolar macrophages which are cleared by mucociliary transport. The re-entry of macrophages into the interstitium is doubted (11,67). Consequently, there is no

established mechanism for formation of interstitial granulomas which contain foreign bodies.

The chemical nature of the dust has a major influence on the fate of phagocytes and, therefore, the capacity of a phagocytized particle to produce disease. A phagocytized particle that is chemically inert may cause lung injury by destroying the phagocyte. This occurs in silicosis and asbestosis. Dead alveolar macrophages release lysosomal enzymes and substances that destroy respiratory epithelium, attract leukocytes and incite interstitial fibrosis (13,108). Particles that persist in alveolar macrophages can evoke sustained secretion of collagenase and elastase (143,144). These alveolar macrhphage secreted proteases can injure respiratory epithelium and incite interstitial inflammation (13). Finally, phagocytized particles can stimulate detrimental immune responses. Organic antigens frequently exert their pathologic effects on the lung via immunologic mechanisms (92,109,116, 140).

The role of particle aspiration from the alimentary tract deserves consideration as a possible mechanism for initiation of plant-particle associated pneumonia in swine. The preceding review of factors that determine if a particular dust can cause disease presents evidence which indicates that particles over 10 µm in nasally inspired air should be efficiently removed by filtration. Whittlestone (147) and Corner and Jericho (27) observed particles up to 50 µm in alveoli. Therefore, it would appear possible that entry of particles into the trachea may originate from a site other than the nasal cavity.

Comparative Human and Nonhuman Species Respiratory Diseases Associated with Inhalation of Organic Antigens

Plant-particle associated pneumonias in humans

In 1946, Dunner et al. reported radiologic evidence of a grain dust associated pneumoconiosis in dock workers who primarily handled grain. The report focused on dock workers who did not have tuberculosis bacteria in their sputa. Clinical signs included chest pain, dyspnea, cyanosis, coughing and rales. Radiologic findings included evidence of nodular infiltration, extensive fibrosis, bronchiectatic cavitation, emphysema and variable degrees of mottling which varied among individuals. Clinical signs did not always correlate with radiologic manifestations. Microscopic and chemical examination of aerial dust from the workplaces revealed wheat, oat, maize, barley and silicates (39). Although an etiologic link between the grain dusts and pneumoconiosis was not fully established, this report did call attention to respiratory problems associated with grain handling. This subject has attracted renewed interest during the last decade (89,136,141).

Head described granulomatous lesions in lungs of 4 patients which had inhaled lentil soup. Two of the patients had been tube-fed lentil soup. A third patient with esophageal carcinoma, sometimes choked and coughed while eating soup. The 4th individual had been fed sieved soup subsequent to an operation for cleft palate. The granulomatous reaction which surrounded particles of about 100 μm in diameter included macrophages, giant cells, and fibrosis. The particles were in bronchioles

and alveoli. Suppurative bronchopneumonia was also widespread in the tube-fed patients. Similar granulomatous lesions were experimentally induced in the subcutis and peritoneal surfaces of mice by lentil soup injections (51).

Subsequent to his finding of 41 cases among approximately 1500 autopsies during a 3-year period, Knoblich proposed that pulmonary nodular granulomatosis, caused by aspirated vegetable particles, was a distinct pathologic entity (64). Cases of vegetable aspiration pneumonia were designated as "lentil pulse pneumonia." His description of the disease also included anatomical details of a lentil, a leguminous plant. The lentil description emphasized the characteristic honeycomb structure of the legume cotyledon. Starch particles within cotyledons ranged from 60 um in diameter for beans to 40 μm in diameter for peas. Knoblich postulated that aspiration of leguminous particles may be of greater importance in pediatrics where artificial soybean formulas are frequently used

Experimental studies in animals were done to characterize the chronologic sequence of the pneumonic process (64). Broth from cooked peas, beans, and lentils was injected intratracheally and directly into the lungs of anesthetized guinea pigs, cats, and rabbits. The animals were killed at various time intervals. Lesions in experimental animals were similar to those found previously in humans. Purulent bronchopneumonia developed within 24 hours after broth injection. Approximately 10 days post-injection, acute pneumonia had subsided and lentil pulses were walled off by giant cells, epithelioid cells and lymphocytes. By

2 weeks post-injection, lentils in granulomas had begun to disintegrate. At two to three weeks post-injection, granulomas often had peripheral fibrosis without capsule formation and some were characterized by fibrous capsules and central macrophages containing digested lentils. Fibrotic nodules and calcified nodules were seen 2 to 3 months after injection. Knoblich observed experimental lentil pulse pneumonia lesions that were structurally similar to lesions of sarcoidosis, tuberculosis, and trichinosis (64).

Vidyarthi described diffuse miliary pulmonary granulomatosis in a human that had been tube-fed. Intrabronchiolar and alveolar granulomas containing vegetable cells 50 μm to 110 μm in diameter were found (137).

Plant-particle associated pneumonias in animals other than swine

White described tissue changes in lungs of 3 calves that aspirated plant material. Calves had been bucket-fed a milk substitute and dry meal. Both feedstuffs contained linseed. Bronchiolar obstruction due to foreign-bodies and an associated granulomatous reaction were seen. Plant material, identified as linseed, was detected in the bronchiolar lesions. Mononuclear cells and a few neutrophils infiltrated linseed particles that occluded a bronchiole. Bronchiolar epithelium was hyperplastic. Older lesions were encapsulated. The honeycombed cotyledon, typical of legumes as described by Knoblich (64) can be seen in the photomicrographic illustrations accompanying White's publication (146).

Saline suspensions of pine pollen, given intranasally to mice and

guinea pigs, evoked an infiltrate of mononuclear cells and neutrophils within 2 days after exposure. Neither giant cells nor granulomas were present at that time; however, granulomas that contained pollen particles formed later and were still visible 3 months after exposure. Guinea pigs inoculated with a lipid extract of pine pollen 12 months before intranasal pollen was given, developed moderate to severe anaphylaxis and an intense chronic inflammation with marked eosinophil infiltration. Hypersensitivity was postulated to play a critical role in development of more extensive chronic lesions (139).

Billups et al. described PAS-positive bodies in pulmonary granulomas that were incidental findings in necropsy specimens from 18 brachycephalic dogs. The honeycombed pattern associated with leguminous cotyledons was not detected. Although these PAS-positive particles resembled starch particles, their identity as plant material was ruled out (7).

Fungi are frequently implicated to cause granulomatous pneumonias. Since fungal antigens have a well-established role in hypersensitivity pneumonias, they will be discussed along with other immune-mediated lung diseases caused by organic dusts.

Immune-mediated lung disease caused by exposure to organic antigens

Several in depth reviews on pulmonary immunopathology have been published (59,60,72,93,115,116,117,118). Coombs and Gell defined 4 types of immune injury mechanisms: Type I (immediate hypersensitivity), Type II (cytotoxicity), Type III (immune complex), and Type IV (delayed hypersensitivity) (26). Research and review articles on immunologic

lung diseases have primarily used the Coombs and Gell classification for discussing mechanisms of immune injury. Recently, Sell extended Coombs' and Gell's classification of immune injury mechanisms to 6 types: (1) inactivation or activation, (2) cytotoxic or cytolytic, (3) toxic complex (Arthus), (4) anaphylactic or atopic, (5) delayed hypersensitivity (cellular), and (6) granulomatous reactions (121).

Inactivation or activation reactions represent mechanisms unrelated to those presented by Coombs and Gell. Inactivation or activation reactions are antibody-receptor interactions that inactivate biologically active molecules, desensitize target cells of biologically active molecules, or sensitize cells to the effects of biologically active molecules. Some examples of immune diseases initiated by inactivation reactions are myasthenia gravis and insulin-resistant diabetes. Hyperthyroidism due to antithyroid receptor antibody is an example of a disease initiated by an activation reaction (121).

Sell subdivided the Coombs and Gell delayed hypersensitivity reactions into delayed hypersensitivity reactions and granulomatous reactions. Delayed hypersensitivity reactions, according to Sell, have T_k (killer) and T_D (delayed hypersensitivity) lymphocytes as immune reactants, and lymphokines and macrophages as accessory components. Delayed hypersensitivity reactions peak in 24 to 48 hours and fade by 3 days. The delayed hypersensitivity reaction kills organisms and virus-infected cells. Mononuclear cell infiltration and target killing of infected cells are pathologic manifestations of delayed hypersensitivity. Representative diseases include viral skin rashes, graft rejection, and demyelination (121).

In contrast to delayed hypersensitivity, granulomatous reactions have T_D lymphocytes as the immune reactant and epitheloid macrophages and giant cells as the accessory components. Granulomas take weeks to develop and months to fade. Infectious agents are isolated but not killed by granulomatous reactions. Tissue replacement by granulomas is the pathologic manifestation of granulomatous reactions (121). Sarcoidosis, berylliosis, and tuberculosis are representative granulomatous reactions (41,121).

The foregoing presentation of classification schemes for immune injury mechanisms provides a basis for discussion of immune-mediated lung disease caused by organic dusts. Immunologic lung diseases have been identified that utilize each of the mechanisms described by Coombs and Gell (26). No immune-mediated lung disease caused by inactivation or activation reactions has been established. Organic dusts are associated with lung diseases mediated by immediate hypersensitivity reactions and granulomatous reactions (115,118,140). Some organic dusts cause lung diseases mediated by more than one type of immune injury mechanism. Grain dusts have been shown to cause pulmonary disease via immediate hypersensitivity and immune complex injury mechanisms (116,141). Multiple immunologic injury mechanisms are initiated by organic dusts that cause hypersensitivity pneumonitis, also called extrinsic allergic alveolitis.

Pepys introduced the term "extrinsic allergic alveolitis" (EAA) to denote those allergic lung diseases which result from inhalation of antigens that participate in immune-complex lung injury (92). The

inciting antigens are usually fungal components, but a few animal proteins can also cause EAA (84,92). Farmer's lung disease is the classical example of EAA (92,110). It is a hypersensitivity pneumonitis affecting primarily agricultural workers (24,34). Natural and experimental animal models of farmer's lung have been reported (15,85,88,91,97,105, 106,149,150,154,159). When moldy hay dust is inhaled, antigens from spores of thermophilic actinomycetes, especially Micropolyspora faeni, are believed to cause formation of toxic immune complexes which initiate reactions that damage the lung (92). However, the immunologic and histopathologic responses that characterize hypersensitivity pneumonitis indicate that both immune complex-mediated and delayed hypersensitivity (granulomatous) reactions occur during the disease process (34,47,101, 104,119,120,140,142). Precipitin and intradermal (Arthus) tests were widely used to provide support for a clinical diagnosis of hypersensitivity pneumonitis, however, it has been demonstrated that neither test correlates reliably with hypersensitivity pneumonitis lesions (36,92, 95,113). Both tests merely indicate prior exposure to antigens (77,94, 142).

Pigeon breeder's disease illustrates the complexity of the immunopathogenesis of hypersensitivity pneumonitis. The histopathologic lesion seen most frequently in lung biopsies is granulomatous pneumonia (52). Immunofluorescent studies of biopsies do not reveal antigen, immunoglobulin or complement (40). Patients with pigeon breeder's disease develop Arthus-like lesions in response to cutaneous injections of pigeon serum (17,44). Both asymptomatic and symptomatic individuals

develop precipitating antibodies against antigens in pigeon droppings (43). Upon challenge with pigeon serum, lymphocytes from patients with pigeon breeder's disease produce migration inhibition factor (MIF) (17,42,77). MIF production is much more closely correlated with the presence of lung lesions than are precipitins (17,45).

The significant similarity between plant-particle pneumonia and hypersensitivity pneumonitis is that both entities are characterized by granulomatous pneumonia with similar histologic features (34,64, 119,120). Plant-particle pneumonia also compares histopathologically with sarcoidosis and berylliosis, diseases mediated by granulomatous hypersensitivity reactions (41,64). Experimental animal models of plant antigen induced granulomatous pneumonia have also been described (152,153,159).

Soybean Characteristics

Nomenclature and seed morphology

Soybean, Glycine max (L.) Merr., is a member of the family Leguminosae, subfamily Papilionoideae (53). Former nomenclatures that have been used for soybean include Phaseolus max (L.), Soja max (L.) Piper, and Soja hispida Moench. Soybean seed structure has been described in detail by Williams (151). The soybean seed contains a hull or seed coat, hypocotyl and cotyledons. The seed coat may be yellow, green, black or any of several shades of brown. A few genetic types of soybean have bicolored seed coats. The yellow seed coat is the major breeding

objective.

Protein bodies and spherosomes are the main organelles identified in soybean cotyledons by electron microscopy (8,111,112,133, 155). Protein bodies may vary from 2 to 20 μm in diameter but usually are in a narrower range of 5-8 μm. Spherosomes or lipid storage bodies are 0.2-0.5 μm in diameter and are interspersed between protein bodies (157). Protein bodies are preserved in defatted soybean flour (133).

Soybean chemical composition

The composition of soybeans varies with variety and location where grown. The average composition of 10 varieties grown at 5 locations was: 42.87% protein, 19.63% oil, 7.98% total sugar, 5.52% crude fiber, and 4.99% total ash. The average iodine number was 128.7 (22).

The principal sugars in soybeans are sucrose, raffinose, and stachyose. Glucose and other reducing sugars are present in substantial amounts in green beans (126). The seed coat contains 9-11% galactomannans, 10-12% acidic polysaccharides, 9-10% xylan hemicellulose, about 40% cellulose, 11% protein and peptides, and the remainder, lignin (4).

Soybean seeds contain a wide variety of amino acids. Methionine is the limiting amino acid in soybean. Glutamic acid and aspartic acid are the predominant amino acids in whole soybean meal, acid-precipitated protein and whey protein. Protein bodies contain at least 60% of the protein in soybean. Tombs' analysis of a defatted soybean flour preparation of protein bodies revealed 82.5% protein, 0.48% total

phosphorus, 1.29% ribonucleic acid, 1.0% phospholipid, 11.3% total lipid, 1.35% phytic acid, and 3.0% carbohydrate (133).

Water, water plus dilute alkali (pH 7-9), and aqueous solutions of sodium chloride (0.5-2 M) are among the most efficient media for extracting soybean proteins (124,125,128). Water extraction is considered as efficient as 1 M sodium chloride extraction of defatted meals (158). Aqueous extracts are usually prepared at room temperature (126).

The components of ultracentrifuge fractions of water-extractable soybean proteins have been reported by Wolf (156). Water-extractable soybean protein contains 2S, 7S, 11S, and 15S fractions that comprise 22%, 37%, 31% and 11% respectively of the total protein. The 2S fraction includes trypsin inhibitors (m.w. 8000-21,500) and cytochrome c (m.w. 12,000). The 7S fraction contains hemagglutinin (m.w. 110,000), lipoxygenase (m.s. 103,000), beta amylase (m.w. 61,700), and 7S globulin (m.s. 180,000-210,000). The 11S fraction contains 11S globulin, the major soybean protein. Glycinin is another name for the 11S globulin. Components of the 15S fraction have not been identified, however this fraction has a molecular weight that is approximately 600,000 (156).

Biologically active components of soybean

Rackis summarized the biologically active components that have been found in soybeans. Principal groups of biologically active soybean components are enzymes, hemagglutinins and proteinase inhibitors. Mature soybean seeds contain amylases, lipases, lipoperoxidase, lipoxygenase,

proteinase and urease (100). Four different soybean hemagglutinins, (A, B, C, and D) have been characterized by isoelectric focusing (23). Mannose and glucosamine are present in all four hemagglutinins. Form B, the major hemagglutinin, contains 4.5% mannose and 1% glucosamine. Soybean whey protein contains the highest concentration of hemagglutinins (100). When soybean hemagglutinin is given parenterally to rats, the LD_{50} is 50 mg per kg. However, 500 mg per kg of soybean hemagglutinin is nonlethal to rats that receive the material via stomach tube (68). Soybean hemagglutinins, like most plant agglutinins, have the ability to agglutinate blood cells of various animal species (56). Hemagglutination and toxicity are apparently associated with the same factor (33,130). Lymphocyte mitogenesis is also evoked by soybean hemagglutinin (123).

Soybeans contain multiple proteinase inhibitors. Kunitz trypsin inhibitor and Bowman-Birk inhibitor are the soybean proteinase inhibitors that have been studied most extensively (100). Kunitz inhibitor, a globular protein, inhibits Kallikrein (138), factor X esterase, thrombin (66) and human serum and plasma trypsins (135). Human pancreatic trypsin is not inhibited by Kunitz trypsin inhibitor (135). Bowman-Birk inhibitor forms a monomer-dimer mixture (49,75). The monomer molecular weight is 8000 (46). Anti-trypsin and anti-chymotrypsin activities are present in Bowman-Birk inhibitor (9).

Raw soybean meal causes pancreatic acinar hypertrophy in rats (98) and is goitrogenic in poorly nourished rats (86). The physiological effects of thyroxine are blocked by an antithyrotoxic factor in soybeans

(107,145). The antithyrotoxic factor is destroyed by proteolytic hydrolysis (87). Antirachitic and growth factors are present in raw or heated soybean meal and their water extracts (18). Conversely, raw soybean meal and isolated soy protein also contain rachitogenic and growth depressing factors (19).

Soybean meal that has been defatted and toasted is commonly used in livestock feeds. Toasting is the process of live steam treatment that destroys the trypsin inhibitors and hemagglutinins. Concurrently, soybean nutritive value is improved (98,99,100). Pancreatic hypertrophy in rats is also decreased by treating soybean meal with moist heat (98, 127).

Soybean allergenicity

Few cases of soybean allergy have occurred in humans and other animals. Allergy attributable to soybean was first reported by Duke. He described a patient who developed asthmatic symptoms upon inhalation of soybean dust or ingestion of diets that included soybean adulterated foods. Four more patients also developed cough and asthma when tested with soybean extract. All 5 patients developed positive skin test responses. Dermal sensitivity to soybean extract was passively transferred to nonsensitive individuals (38).

Subsequently several reports have described human occupational asthma and neonatal gastrointestinal allergy due to soybean exposure (10,16,25,73,78,79,90,148). Ratner et al., however, had concluded that soybeans are weakly antigenic in humans (102). Ratner and Crawford also

considered soybeans to be only weakly antigenic in guinea pigs (103). Perlman reported that soybean extracts heated at 100 C for 30 minutes elicited strong positive skin reactions in humans, but heating at 180 C for 30 minutes virtually prevented the skin reaction (96).

Barratt et al. (6) and Kilshaw and Sissons (61) described anaphylactic type gastrointestinal hypersensitivity to soybean protein in preruminant calves fed soybean-containing milk replacer. Kilshaw and Sissons determined by immunoelectrophoresis that β-conglycinin was the soybean component responsible for the gastrointestinal hypersensitivity in preruminant calves. IgE antibodies were detected by passive cutaneous anaphylaxis testing on a 7 week-old calf (62). Ileal villous atrophy with lamina propria edema and lymphocytic infiltration was seen in hypersensitive calves (6). The lesions in the intestine of calves are histologically similar to lesions in the duodenum of soybean-hypersensitive human infants (2,6).

The preceding literature has presented a summary of environmental diseases of swine, with special attention given to the effects of dusts on swine health. In view of what is known about the effects of organic dusts on mammalian lung structure and immunologic response characteristics, discussion of soybean structure, chemistry, and biological activity including allergenicity was also deemed pertinent background information for the studies reported in this dissertation.

PART I. NATURALLY OCCURRING PLANT-PARTICLE ASSOCIATED PNEUMONIA OF SWINE

This manuscript will be submitted to Veterinary Pathology

ABSTRACT

The incidence and lung lesion characteristics of naturally occurring plant-particle associated pneumonia of swine were determined by retrospective histologic examination of lungs from 62 confinement-reared, self-fed pigs; 444 pig necropsies accessioned during a 5 year period; and gross and histologic examination of the lungs of 26 confinement-reared, floor-fed pigs.

Twenty-five of 62 self-fed pigs (40.3%) from the same herd were found to have granulomatous lesions containing plant particles. Another 15 pigs (24%) from the same group had similar granulomatous lesions without identifiable plant material. Plant material was found in 9 of 18 pigs that had granulomatous pneumonia among the 444-pig group. Plant material was not found in lungs of floor-fed pigs. Particles morphologically consistent with soybean were found in 7 of 25 self-fed pigs and 6 of 9 pigs among the 444-pig group.

Lung lesions were predominantly granulomatous. Plant particles 20 to 100 μm were free in alveoli, or within macrophages, multinucleated giant cells and interstitial granulomas. Neutrophils were admixed with macrophages and giant cells in alveoli and bronchioles of consolidated lung. Obstructive suppurative and granulomatous bronchiolitis was a feature of the spontaneous disease.

Aspiration of particles from the oral cavity was considered the most likely initiating event leading to plant particle-associated

pneumonia. Self-feeding may be a predisposing factor to plant-particle associated pneumonia of swine.

INTRODUCTION

Naturally occurring pneumonia caused by aspiration of vegetable material has been reported in calves (21) and pigs (3,11,25). Particles of linseed up to 600 μm in diameter caused bronchiolar obstruction in bucket-fed calves (24). Lungs of pigs fed a dusty mixture of peas, wheat and barley frequently contained intra alveolar plant particles 15 μm in diameter. During a 2 year period, 40 cases of cereal-particle associated pneumonia occurred among 68 pigs used as test or control pigs in a respiratory mycoplasmosis experiment. Examination of lung sections from 4 uninoculated control pigs revealed particles up to 300 μm in bronchioles and up to 50 μm in alveoli. The inflammatory response to plant particles included alveolar accumulation of neutrophils, macrophages and giant cells, and granuloma formation. In bronchioles with plant particles, there were neutrophil accumulation, bronchiolar epithelial destruction and hyperplasia, and replacement of epithelial cells by connective tissue (3).

Factors responsible for plant-particle associated pneumonias in pigs have not been clearly defined. Published reports indicate that most dust in enclosed swine facilities is derived from feed, bedding or solid excreta (2,5,13,25). Fifty to ninety-five percent of airborne dust particles in enclosed swine units are less than 5 μm (2,4,13). Consequently, they may be inspired into alveoli (1,8). Swine-house dust has been associated with an increased incidence of pneumonia (2,13,14). However, dust particles under 5 μm are believed to have no significant direct effect on swine lung structure (4,6,10). In pigs, most inhaled

particles over 5 μm are transported by the nasal cavity mucociliary apparatus to the pharynx where they are swallowed (21).

Few reports discuss the role of feeding practices in the epizootiology of plant-particle associated swine pneumonia. Powdered rations had no positive tendency to induce aspiration pneumonia (16). Two reports of plant-particle associated swine lung lesions indicated that troughs and self-feeders were used in the affected herds (3,11).

This report describes plant-particle associated lung lesions in pigs fed pelleted corn-soybean rations from self-feeders and automatic drop floor-feeders. Findings of a retrospective histopathologic survey to determine incidence and characteristics of plant-particle associated lesions in pig lungs from 444 pig necropsies are also presented.

MATERIALS AND METHODS

Lung sections from two groups of pigs were examined by light microscopy to determine the characteristics and incidence of spontaneous plant-particle associated pneumonia. The lungs of a third group of pigs were grossly and histologically examined.

Group 1 included lung sections from 62 market weight (approximately 105 kg) pigs from an Iowa commercial herd. The pigs had been used in a field trial to test efficacy of a chemotherapeutic agent. The pigs had been obtained in Kentucky when they weighed approximately 7 kg each. They were housed in a 20 x 14 m building until slaughtered approximately 5 months later. Building temperature was maintained at or near 26.6 C during the cold season by forced air heat. Ventilating fans with flow rates of 1800 to 6000 cfm, supplemented by natural ventilation during warmer periods, provided adequate ventilation. Ten pigs per group were reared in 6.1 x 4 m pens. Each pen had a concrete floor except for the rearmost 1.2 m which had a wooden slotted floor. A single, height-adjustable, nipple-type waterer was mounted to a pen partition above the slotted floor. Animal waste deposited into a pit beneath the slotted floor was aerated by recirculated water that had been injected with compressed air. A 2-port self-feeder was used in each pen to feed a pelleted 14% protein finishing ration. Vegetable particulates in the feed consisted of corn and 44% protein, defatted soybean meal. Meat and bone scraps, minerals and vitamins were also in the feed. The herd history indicated that clinical signs of pneumonia were rarely observed.

Lung specimens from apical and cardiac lobes, and grossly abnormal sites of other lobes were collected at slaughter and submitted to the Veterinary Medical Research Institute (VMRI) at Iowa State University for mycoplasmosis immunofluorescence testing and histologic examination.

Group 2 consisted of 26 market weight pigs from a 700-head finishing unit at Iowa State University. The finishing unit was 37.5 x 14.5 m. Pigs were housed in groups of 9 to 24 in 1.4 x 6.7 m pens or up to 30 in 3 x 6.7 m pens. A fixed-height, nipple-type waterer was mounted 36 to 45 cm above a 5 cm deep by 1 m wide dunging area at the rear of each pen. The dunging area was periodically flushed by water that drained to a lagoon. A corn-soybean diet similar to that fed to group 1 animals was deposited on the pen floor from at least 2 automatic feeder ports 1.9 m above. Ventilating and heating conditions were the same as those for group 1 pigs. Lungs from these pigs were collected at slaughter and stored in an insulated chest until grossly examined 3 hours later. Specimens of normal lung tissue, and abnormal tissue when present, were collected from each lung lobe for histologic examination.

Group 3 consisted of lung sections from 444 pig necropsies performed at the VMRI from 1976 through 1981. The pigs were from Iowa commercial herds, Iowa State University herds and experimental animal groups used at the VMRI. These pigs had been reared in a variety of open and enclosed facilities. Feeding practices varied; however, most pigs received pelleted feed similar to group 1 and 2 pigs.

All tissues examined microscopically had been fixed in 10% neutral-buffered formalin and processed by routine methods for light microscopy. Lung sections from each pig were stained with hematoxylin and eosin. Additionally, duplicate sections from pigs in Group 1 were stained with Giemsa stain, and periodic acid-Schiff reagent without diastase. Soybean particles were identified on the basis of their leguminous cotyledon structure (22,26).

RESULTS

Lung sections from 40 of 62 pigs in group 1 had granulomatous lesions. Twenty-five pigs among the 40 with granulomatous lesions also had plant material in lung lesions. Soybean cotyledons were indentifiable in lung lesions of 7 pigs. Cotyledons contained elliptical thick walled cells, usually 20 x 25 μm, that were arranged in a honeycomb-like cluster. Cotyledon cell walls were translucent, yellow to white, and anisotropic in hematoxylin and eosin stained sections. Cytoplasm, which consists mainly of protein bodies, contained amorphous and globular eosinophilic material. Cytoplasm was either weakly stained or unstained by periodic acid-Schiff reagent, whereas, the cell wall was stained intensely.

Plant particles varied in size and shape. Soybean cotyledons and other plant materials from 20 to 100 μm were either free or within macrophages and multinucleated giant cells in alveolar spaces. The cytoplasm of cotyledon cells was frequently infiltrated by neutrophils. Neutrophils were also admixed with macrophages and multinucleated giant cells in more severely consolidated lung lesions (fig. 1). Elongated particles up to 300 μm were occasionally seen in alveoli (fig. 2). Changes in the alveolar wall were variable but included alveolar collapse, pneumocyte degeneration, pneumocyte hyperplasia, interstitial edema, and infiltration by macrophages, lymphocytes and plasma cells. Interstitial fibroplasia and fibrosis occurred alone and in association with interstitial granulomas. Granulomas had central multinucleated giant

cells which sometimes contained plant remnants.

Terminal bronchioles, 140 to 260 μm in diameter, were frequently occluded by plant particles or aggregates of giant cells and macrophages. Bronchiolar epithelium adjacent to plant particles was usually attenuated if not completely denuded. Longitudinal sections of occluded terminal bronchioles had foci of epithelial hyperplasia near the occlusion. Only the smooth muscle layer remained in severely affected bronchioles. The inflammatory response was variable in bronchioles. When soybean cotyledons with cytoplasmic matrix were present, neutrophils were frequently predominant and epithelial changes were less severe. When particles were degraded or bronchiolar epithelium was desquamated, macrophages, multinucleated giant cells, fibroplasia and fibrosis were more prevalent (fig. 3,4). Lymphoid aggregates associated with bronchioles were frequently hyperplastic.

Seven pigs in group 2 had dark red contracted firm tissue in the entirety of the bronchopulmonary segments or in isolated foci of one or more apical and cardiac lobes. Intermediate lobes and anteroventral portions of diaphragmatic lobes occasionally had similar focal lesions. Twenty-four of 26 pigs had mild granulomatous pneumonia characterized by multifocal aggregates of macrophages in alveolar spaces and interstitium. Sections from grossly abnormal lung lobes usually had atelectic lobules with marked interstitial infiltration by macrophages. No plant particles were found in lung sections of any group 2 pig. Marked lymphoid hyperplasia that included prominent germinal centers and paracortical zones occurred in the bronchiolar associated lymphoid tissues and

tracheobronchial lymphoid nodes of 25 of the 26 pigs. Goblet cell and mucous gland hypertrophy was occasionally observed in the larger bronchioles.

Twenty-three of 444 pigs in group 3 had granulomatous lung lesions. Six of the 23 had identifiable soybean cotyledons in the lesions. Three other pigs had plant material in their lungs. Lesions in this group of pigs were usually discrete densely fibrosed interstitial granulomas (fig. 5,6). One granuloma consisted of lymphocyte and macrophage infiltrated connective tissue around a 228 x 118 μm cluster of soybean cotyledon cells (fig. 7,8).

DISCUSSION

Twenty-five of 62 pigs (40.3%) from one herd were found to have granulomatous lung lesions containing plant particles. Another 15 pigs (24%) from the same group had similar granulomatous lung lesions without identifiable plant material. A high incidence of plant-particle associated pneumonia in a single herd, as found in this study, has been described (3). Our findings suggest that plant-particle associated pneumonia may be more common than would be suggested by the few reports (3,11,25).

Between 2 groups of pigs, soybean particles were found in 28% (7 of 25) and 66.6% (6 of 9) pigs with plant-particle associated pneumonic lesions. To our knowledge, this is the first report to confirm presence of soybean particles in swine plant-particle associated pneumonic lesions. The actual incidence of soybean particles in lungs of group 1 pigs may have been higher if more sections from each lung had been examined. The characteristic soybean cotyledon structure readily distinguishes it from other plant materials in corn-soybean meal swine rations. Because of its availability, high protein quality and concentration of protein, soybean is the most frequently used legume in Iowa swine rations. Particles from a mixture of peas, barley and wheat were previously associated with swine plant-particle pneumonia lesions (11).

The lesions we observed in lungs of pigs with plant-particle pneumonia were similar to granulomatous lung lesions reported previously

in pigs (3,11,25). Neutrophils were often admixed with macrophages in alveoli or within and around soybean cotyledons. Their presence in the cytoplasm (protein bodies) of soybean cotyledons suggest a chemotactic response. Neutrophils may also be present because of concurrent bacterial infection. Acute bronchopneumonia has been previously described as a complication of swine plant-particle associated pneumonia (25).

The findings of soybean in lung lesions of several pigs indicates a need for more studies on the effects of soybean particles on swine lung structure. The possible interaction of soybean particles with infectious respiratory pathogens also needs to be determined. Further studies on plant-particle associated pneumonia in swine are indicated to characterize epizootiologic factors, clinical signs, gross lesions, and effects on swine performance.

Soybean or other plant particles were not found in lung lesions of group 2 pigs. Additionally, lesions observed did not consist of giant cells and interstitial granulomas. Therefore, an important qualitative difference existed in lung lesions of group 2 compared to groups 1 and 3 pigs. The marked lymphoid hyperplasia seen in lungs and tracheobronchial lymph nodes of group 2 pigs is morphologically consistent with lesions attributed to Mycoplasma hyopneumoniae infection (15,19).

Soybean particles appear to be significant in development of plant-particle associated pneumonic lesions. The lesions we describe are similar to those described for "lentil pulse pneumonia," a human

disease caused by aspirated lentils, peas, and beans (9,12) or other vegetable materials (23). Lentil pulse pneumonia was established as a distinct pathologic entity after several autopsy cases of lung disease, including bronchopneumonias, originally attributed to bacteria, were found to have lentil pulse particles and multinucleated giant cells on microscopic examination. Lentil pulse pneumonia lesions have been produced experimentally in cats, rabbits and guinea pigs (12). The leguminous lentil cotyledon structure is similar to soybean cotyledon structure.

Swine plant-particle associated pneumonia lesions are also similar to granulomatous lung lesions seen in sarcoidosis, berylliosis (7), and the granulomatous form of farmer's lung (17,19). These are human immune-mediated granulomatous respiratory tract diseases.

In this study, plant-particle associated pneumonic lesions were found in self-fed but not floor-fed pigs. Since the method of feeding was the most relevant difference between pigs in groups 1 and 2, self-feeding is considered the most likely environmental factor predisposing to plant-particle associated pneumonia in pigs. Previous reports indicate occurrence of lesions in lungs of trough-fed pigs (3) and self fed pigs (11). Plant-particle associated pneumonia also occurred in floor-fed pigs which had previously been self-fed (11).

Aspiration of food particles from the oral cavity is the most likely initiating event leading to plant-particle associated pneumonia in pigs. Previous reports indicate swine (21) and humans (17) can effectively remove most inhaled particles over 5 μm by nasal filtration

and mucociliary transport of particles from the nasal cavity to the pharynx. Since the particles identified in this and previous studies (3,10) have been larger than 5 μm, inhalation would appear unlikely as the means by which particles enter the respiratory tract. It may also be possible that swine with severe atrophic rhinitis could inhale particles over 5 μm which would reach the alveoli. Aspiration during feeding has been reported as the means by which bucket-fed calves developed plant-particle associated pneumonic lesions (24).

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Fig. 1: Neutrophils, macrophages and multinucleated giant cells are admixed in alveoli of a self-fed pig with spontaneous plant-particle associated pneumonia lesions. Remnants of plant material can be seen in the multinucleated giant cells. HE stain. Bar = 50 μm.

Fig. 2: Plant-particle with prominent cell walls in an alveolus of a self-fed pig. Macrophages are along the periphery of the plant-particle. The entire particle was 308 μm long. HE stain. Bar = 20 μm.

Fig. 3: Obstructive bronchiolitis and bronchopneumonia in a self-fed pig. The cell wall of the soybean cotyledon in the bronchiolar lumen is PAS positive. The mucosal epithelium is effaced. Neutrophils and macrophages are in the bronchiolar wall, interstitium, alveoli, and adjacent bronchioles. The arrow points to the thin smooth muscle layer identifying the bronchiolar wall. PAS stain. Bar = 50 μm.

Fig. 4: Multinucleated giant cells and macrophages fill the lumen of a bronchiole in the lung of a self-fed pig. Bronchiolar epithelium has been destroyed. The bronchiolar smooth muscle (arrow) remains. Macrophages and multinucleated giant cells are predominent cells in the alveolar exudate and within fibroplastic interstitial connective tissue. HE stain. Bar = 50 μm.


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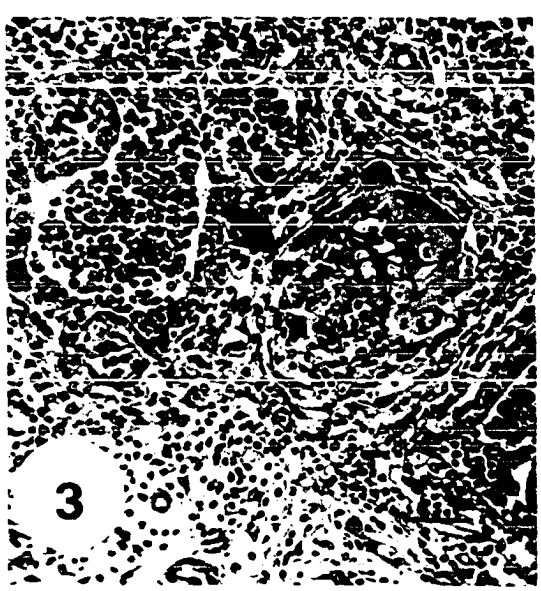
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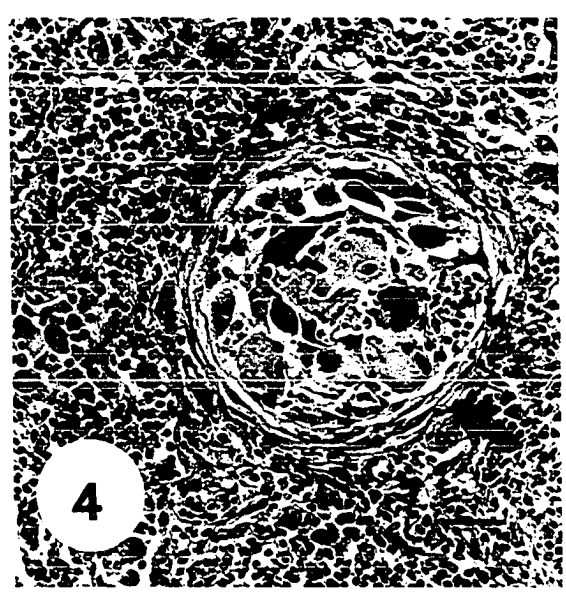
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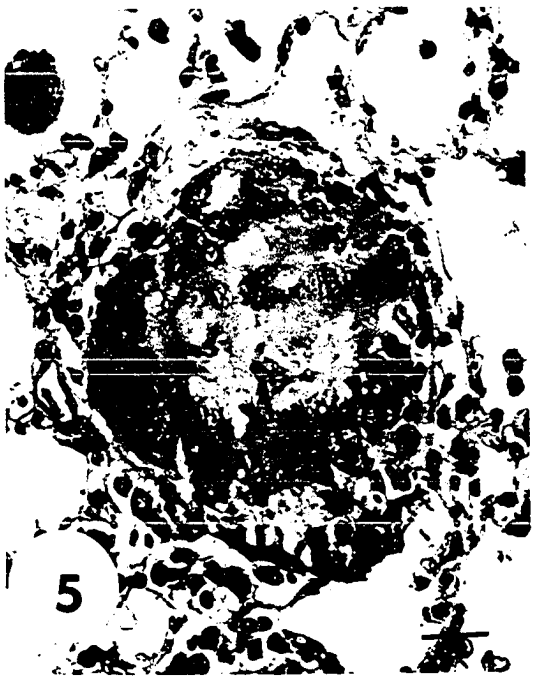
Fig. 5: Interstitial granuloma in a pig from group 3 that had soybean in its lung. A Langhans type multinucleated giant cell is surrounded by a thin connective tissue zone. Alveolar epithelium of adjacent alveoli overlie the connective tissue which has enclosed the giant cell. Remnants of plant material are in the giant cell cytoplasm. HE stain. Bar = 20 μm.

Fig. 6: Interstitial granuloma with marked peripheral fibroplasia in lung from a group 3 pig. Macrophages, a few neutrophils and lymphocytes are in the connective tissue. A few macrophages are in adjacent alveoli. A partially decomposed portion of soybean cotyledon is in the multinucleated giant cell cytoplasm. HE stain. Bar = 50 μm.

Fig. 7: Soybean cotyledon obstructed bronchiole in a group 3 pig. There is marked peribronchiolar lymphoid and macrophage infiltration and dense collagen fibers. The inflammatory cells extend into the lamina propria of adjacent bronchioles and into the interstitium of adjacent alveoli. Neutrophils are in the soybean cotyledon cell cytoplasm. HE stain. Bar = 50 μm.

Fig. 8: Anisotropic cotyledon cell walls of soybean particle in fig. 7 have characteristic honeycomb structure of legume cotyledon. Polarized light photomicrograph of HE stained section. Bar = 50 μm.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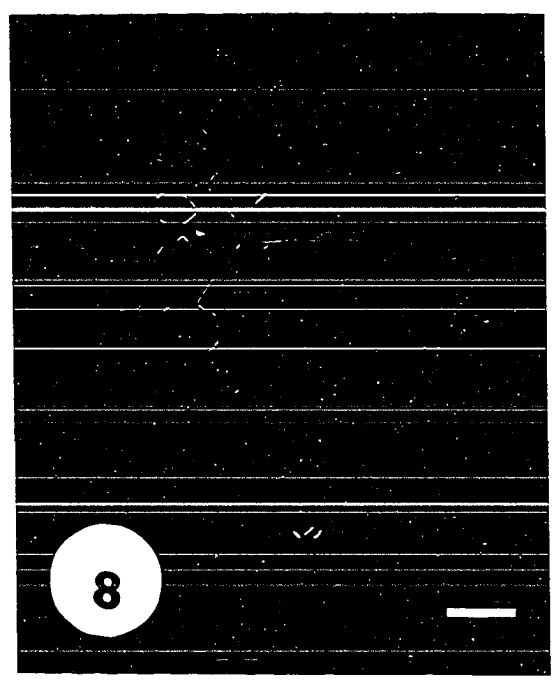
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PART II. EXPERIMENTAL SOYBEAN-INDUCED PLANT-PARTICLE PNEUMONIA OF SWINE

This manuscript will be submitted to Veterinary Pathology.

ABSTRACT

Clinical signs and effects of soybean particles on swine lung structure were studied in 55 soybean-treated and 19 control conventional pigs that were killed 7, 14, or 21 days after transtracheal inoculation. Each animal received one dose of soybean or saline. Effects of dose volume, soybean concentration (% by volume), sterilization, sterilization method, and plant particle size were concurrently studied in order to determine a dose regimen for consistent reproduction of plant-particle pneumonia in experimental pigs.

Coughing, which lasted up to 2 hours, occurred after soybean administration to 6 pigs that received 20 ml of 10% soybean particles. This dose contained the highest amount of particles given any animal. One soybean-treated pig and one control pig died within one hour after inoculation. Lung rupture was found in the control pig. No lesions were seen in the soybean-treated pig. One pig that received 20 ml of 10% soybean had a bullous cavity in the right diaphragmatic lobe. This was the only gross lesion attributable to soybean treatment.

Microscopic lesions morphologically consistent with plant-particle pneumonia were found in 35 of 55 soybean-treated pigs. Sparse multifocal aggregates of macrophages and multinucleated giant cells in alveoli, focal interstitial granulomas and soybean obstructed bronchioles were the lung lesions found in soybean-treated pigs. Small bullous foci, hematomas and intraalveolar hemorrhage were other microscopic lesions seen in pigs

receiving the largest dose of soybean particles. Larger particles over 265 µm primarily caused bronchiolar obstruction. Smaller particles (under 105 µm) caused both bronchiolar obstruction and alveolar lesions. Neither sterilization nor sterilization method had an important influence on characteristics of the experimental disease.

Lesions seen in the experimental disease were not as extensive or as severe as those in the natural disease. However, the results demonstrate that soybean particles can cause clinical respiratory disease, gross and microscopic lung lesions in swine.

INTRODUCTION

Plant-particle associated swine pneumonia has been reported previously (1,5,13,15); however, few reports have established the effect of specific plant materials on the lung. Lung structure was not affected in pigs exposed for 6 weeks to aerosolized cornstarch particles 5 μm or less in diameter (2). Although pigs showing retarded growth had more starch particles in their lung than other pigs, powdered rations had no tendency to induce aspiration pneumonia (8). Starch particles found in lungs of vitamin A-fortified pigs and vitamin A-deficient pigs were given little significance in pneumonic processes (4).

Plant material that included indentifiable soybean particles was associated with spontaneous granulomatous pneumonic lesions in 25 of 62 self-fed pigs from an Iowa commercial herd. Soybean particles were also found in granulomatous lesions from 6 of 9 cases of plant-particle associated pneumonia among 444 pig necropsies surveyed retrospectively for evidence of plant-particle associated pneumonia. Plant particles, including soybean cotyledons, from 20 μm to 100 μm were free in alveoli or in macrophages and giant cells, and in interstitial granulomas. Bronchioles up to 260 μm in diameter were occluded by soybean cotyledon and other plant particles. Granulomatous inflammation was the predominant tissue reaction, although neutrophils were admixed in alveoli of more severely consolidated lung sections.

Neutrophils also had a tendency to infiltrate the cytoplasmic compartment (protein bodies) of soybean cotyledon cells (13).

Lesions of plant-particle associated pneumonia in pigs resemble lesions in bucket-fed calves caused by aspirated milk substitute and meal containing linseed particles (14), and human pulmonary lesions caused by aspirated lentils (3,6), or other vegetable materials (11).

This report presents gross and histologic findings from conventional and respiratory disease free pigs transtracheally given aqueous or saline soybean suspensions in an attempt to reproduce lesions occurring in natural swine plant-particle associated pneumonia (1,5,13,15). Our objectives were to: 1) determine effect of soybean particles on swine lung structure; 2) determine if clinical signs of illness occurred in pigs that received soybean transtracheally; and 3) establish the dose regimen required to reproduce lesions consistent with those seen in spontaneous plant-particle associated pneumonia.

MATERIALS AND METHODS

Five experiments were conducted to: 1) determine effects of trial doses of soybean particles (exp A); 2) determine effects of dose volume, percent soybean particles and necropsy interval (exp B); 3) compare effects of autoclaved versus ethylene oxide sterilized soybean particles (exp C); 4) compare effects of different soybean particle sizes (exp D); and 5) compare effects of sterile versus nonsterile soybean particles on clinical condition and lung structure of swine (exp. E). Treatments, pigs per treatment and necropsy intervals are shown in Table I.

Pigs from Iowa State University's Laboratory Animal Resources (LAR) herd were used in experiment A. This herd was established from Caesarean derived breeding stock and has been maintained in an infectious disease barrier unit. Pigs in this herd are free of respiratory infections caused by the major swine respiratory pathogens: Mycoplasma hyopneumoniae, M. hyorhinis, Bordetella bronchiseptica, Pasteurella multocida and Haemophilus spp. The herd is monitored for infectious diseases by periodic microbiological and serological examinations, and by necropsy examination of experimental pigs. Four groups of conventional pigs were used in the other experiments. One or more intact litters were used in each experiment. Pigs from each litter were randomly assigned to each treatment. This tended to disperse pigs with similar weights more uniformly among treatments. Pigs from the LAR herd averaged 10.2 kg bodyweight. Conventional pigs

used in experiments B, C, D and E had bodyweight averages of 10.2, 13, 18.8 and 24.1 kg respectively.

Pigs were housed in groups of 12 or less in 2.8 x 2.8 m or 3.0 x 3.2 m isolation units where the temperature was maintained at or near 26.6 C during an experiment. Radiated heat from hot water was used during cold periods. Each unit was ventilated by a 3000 rpm, 1/60 hp rotary blower and a 0.3 x 0.3 m filtered ceiling vent. Each unit was cleaned daily with pressurized water immediately before feeding.

Pigs were floor-fed a pelleted 14% ration that included corn and soybean meal. No antibiotics or other growth promotants were in the feed. Water was provided ad libitum in a floor-level, bowl type waterer that refilled when a pig pressed its snout against the water control lever.

Pigs were placed in isolation units at least 3 days before experiments were started. Each pig was observed daily for signs of illness during the acclimation period.

Inoculum was prepared immediately prior to use or a few days in advance and refrigerated until used. Defatted, 44% protein soybean meal was ground at high speed in an electric blender (Osterizer, Model 864, John Oster Mfg., Milwaukee, Wisconsin). Aerosolized particles that impinged upon the glass sides of the blender flask were transferred with a teaspoon to a plastic 0.2 or 0.4 l container which was then sealed. Soybean particles used in experiment D were separated with a series of sieves having mesh sizes of 25, 35 and 45 (Cat. No. 37845,

Bel-Art Products, Pequannock, New Jersey). Particles not retained by the smallest (45-mesh) sieve and particles retained by the 45-mesh and 35-mesh sieves were collected and designated sizes 1, 2, and 3 respectively. A sample of each fraction was dry-mounted on a microslide. A calibrated light microscope eyepiece micrometer was used to measure 21 large particles on each slide. Average particle sizes were 69 x 105 μm for size 1, 263 x 265 μm for size 2, and 360 x 499 μm for size 3. Particles as small as 3 μm were in each sample. Soybean particles were either sterilized with ethylene oxide gas or suspended in saline or deionized distilled water and autoclaved at 115 C and 1.75 kg/cm^2 absolute pressure for 20 minutes. Sterile saline or sterile deionized water was used to adjust soybean concentration of test inoculum. The percent of solids by volume was determined by averaging the packed volume of 2 samples in microhematocrit tubes centrifuged for 5 minutes (microhematocrit centrifuge, Model No. CT-2900, Clay Adams, Inc., New York, New York). When the desired concentration was obtained, the inoculum was sealed in 100 ml vaccine bottles until used. Sterile saline and sterile deionized water for treatment of control pigs was also stored in sealed vaccine bottles until used.

Prior to administration of the test or control dose, each pig was sedated with an intramuscular injection of 1 ml/2.7 kg body-weight (up to 4 ml total) of 100 mg/ml ketamine hydrochloride (Ketaset, Bristol Laboratories, Syracuse, New York) that had 1 mg/ml acepromazine maleate (Ayerst Laboratories, Inc. New York, New York) added. The pig to be inoculated was placed in dorsal recumbency on a v-shaped wooden rack

1 m above the floor. Nasal secretions from one nostril were collected on a calcium alginate swab (Calgiswab, Inolex, Glenwood, Illinois) and stored in a plastic tube containing 0.2 ml tryptose phosphate broth containing 1:2000 bacitracin. A 6 ml blood sample was collected by vena cava puncture. Inoculum was administered transtracheally from a 20 ml syringe with a 5 cm 16 gauge needle. Following inoculation, the pig was elevated to a vertical position and tilted to the right.

Pigs were observed twice daily for up to 3 days postinoculation and once daily thereafter for signs of clinical illness. Body temperatures were measured with an electronic probe thermometer (DIGI-SENSE, Model 8520, Cole Parmer Instrument Co., Chicago, Illinois) and recorded.

When an experiment was terminated, pigs were electrocuted and exsanguinated. A 20 ml blood sample was collected during exsanguination. At necropsy, the trachea was exposed in the cervical region. A sterile cotton-tipped swab (Cat. No. A5002-5, Scientific Products, McGaw Park, Illinois) was passed through a heat sterilized incision 1 cm caudal to the larynx to collect tracheal fluids for microbiology. After the tracheal fluids had been collected, the lumen was tied closed with twine and the head removed. In situ lungs of all pigs except those used in experiment A were filled transtracheally with 10% neutral-buffered formalin from a reservoir 1 m above the thorax. Five minutes was usually an adequate filling time; however, observation and palpation of the lung through the abdominal surface of the diaphragm provided a better indication of the degree of lung distension. The in situ fixed lung and trachea

were removed from the thorax, examined grossly, photographed and placed in 10% neutral-buffered formalin in a 114 1 plastic storage container until trimmed for histologic processing. Portions of tracheobronchial lymph node, caudal cervical lymph node, mesenteric lymph node, liver, spleen, and kidney were also collected and fixed in neutral-buffered formalin for histologic processing. The snout was transected at the level of the first premolars and examined for evidence of atrophic rhinitis. The head was bisected longitudinally and a sample of nasal secretion aseptically collected from mucosa of the ethmoid conchae. Gross alterations were recorded during necropsy.

Formalin fixed tissues were processed by routine methods for light microscopy. Abnormal tissue, when present, and grossly normal tissue were collected from each lung lobe for histologic processing. Usually sections stained with hematoxylin and eosin were examined; however, duplicate lung sections stained with periodic acid-Schiff reagent, and Giemsa stain were occasionally examined. Soybean particles were identified on the basis of their cotyledon structure (10,16). The character and extent of microscopic lesions were recorded when observed. Representative lesions were photographed.

Serum from blood collected at inoculation and necropsy was tested by a microtiter complement-fixation method (9) for antibodies against M. hyopneumoniae and M. hyorhinis. Secretions collected at inoculation were cultured for 48 hours on 5% horse blood agar plates diametrically streaked with Staphylococcus epidermidis, and MacConkey's dextrose agar

to identify colonies of the common swine respiratory bacterial pathogens: B. bronchiseptica, P. multocida and Haemophilus spp.

RESULTS

Coughing occurred immediately after soybean administration to pigs used in experiment E but subsided within 2 hours. Clinical signs were not observed in other pigs. No pigs had febrile responses. One control pig in experiment B and one autoclaved soybean-treated pig in experiment C were found dead within one hour after inoculation. At necropsy, diffuse pulmonary and intrathoracic hemorrhage was observed in the control pig. The autoclaved soybean-treated pig had no lesions.

Pulmonary gross lesions were found in 3 pigs killed at predetermined postinoculation intervals. Focal pleural fibrosis with adhesion of costal and pulmonary pleura was observed in one control pig in experiment D and one sterile-soybean-treated pig in experiment E. Another sterile-soybean-treated pig in experiment E had a well-demarcated bullous cavity, approximately 6 cm long by 3 cm in diameter, in the right caudodorsal portion of the diaphragmatic lobe. The cavity was hollow and coated by a thin film of coagulated blood. Either unilateral or bilateral mild to moderate atrophy of the ventral scroll of the ventral turbinates was observed in 4 conventional pigs. The only gross lesions found outside the thorax were unilateral and bilateral renal cysts in 5 experiment B pigs which were littermates. Three of the pigs with renal cysts had received soybean while the other 2 had received sterile distilled water.

Microscopic lung lesions were found in 35 soybean-treated pigs and 1 control pig but none of these pigs had gross lesions. In experiment

A, all 4 pigs had mild alveolar lesions consisting of scattered interstitial granulomas, occasional macrophage aggregates and giant cells in alveoli, and alveolar epithelial hyperplasia. Alveolar wall thickness was exaggerated because the immersion fixed lung contracted before being formalin-fixed. No lesions were observed in the control pig.

In experiment B the highest incidence of microscopic lung lesions was in 5 of 6 pigs killed 7 days after receiving 10 ml of either 10, 5 or 2.5% autoclaved soybean. The pig without lesions in the latter group had received 10 ml of 10% autoclaved soybean. One pig in each pair of pigs killed 7 days after receiving 5 ml per pig of either 10, 5 or 2.5% autoclaved soybean had microscopic lesions. At 21 days postinoculation, microscopic lung lesions were observed in both pigs receiving 10 ml of 10% autoclaved soybean and both pigs receiving 5 ml of 10% autoclaved soybean. One pig in each pair of pigs killed 21 days after inoculation of 10 ml of 5% or 2.5% autoclaved soybean, or 5 ml of 2.5% autoclaved soybean had microscopic lesions. The only soybean-treated group having no pigs with lesions was a group of 2 pigs killed at 21 days after receiving 5 ml of 5% soybean. One control pig killed 21 days postinoculation had a focal alveolar aggregate of macrophages with a few admixed neutrophils. No microscopic lesions were seen in other control pigs (fig. 1).

Lesions were sparse in affected lung sections of soybean-treated pigs (fig. 2). No pig had more than 4 lung lobes with lesions. Macrophage aggregates and multinucleated giant cells in alveoli were the predominant alveolar alterations (fig. 3,4). Occasional interstitial

granulomas with and without plant material were seen in pigs killed at 21 days (fig.5).

Frequently, a soybean cotyledon-obstructed terminal bronchiole was the only alteration found in an affected lung lobe. Soybean particles 150 to 360 um in diameter were found in obstructed bronchioles. Bronchiolar epithelium at the periphery of the soybean particle was usually attenuated or focally eroded. Neutrophils, macrophages and giant cells were along the periphery of plant particles and in the bronchiolar lamina propria. Peribronchiolar connective tissue was infiltrated by neutrophils and macrophages. Cytoplasm of soybean cotyledon cells was infiltrated by neutrophils. Bronchiolar-obstructing soybean cotyledon fragments contained more neutrophils when pigs were killed at 7 days postinoculation than at 21 days (fig.6,7,8). The bronchiolar wall around an obstruction was fibrosed in some pigs killed at 21 days postinoculation.

In experiment C, two pigs receiving autoclaved soybean and 2 pigs receiving ethylene oxide-sterilized soybean had microscropic lesions that were similar in extent and character to those in experiment B. Fewer neutrophils were observed at 14 days than were seen at 7 days in experiment B pigs. No control pigs had lesions.

Three pigs receiving size 1 particles, 4 pigs receiving size 2 particles, and 1 pig receiving size 3 particles in experiment D had microscopic lesions. Scattered bronchiolar obstruction caused by soybean cotyledons was the predominant lesion in all affected pigs. The inflammatory response consisted of macrophages within and around soybean

cotyledons, bronchiolar epithelial attenuation and erosion, fibrosis and macrophage infiltration in the lamina propria and peribronchiolar fibroplasia and infiltration by macrophages and lymphocytes. Less commonly focal alveolar aggregates of giant cells and macrophages were observed in lung sections of each pig with bronchiolar lesions. Alveolar lesions were most numerous in pigs receiving the smallest (size 1) particles. One control pig had focal pleural fibrosis. No lesions were observed in lung sections of other control pigs.

Microscopic lesions were observed in 3 of 3 pigs receiving ethylene oxide sterilized soybean particles and 2 of 3 pigs receiving nonsterile soybean particles in experiment E. No control pigs had lesions. A focus of pleural fibrosis was observed in one sterile soybean-treated pig with grossly recognizable pleural fibrosis. Two pigs from the same treatment group had hemorrhagic subpleural and interlobular bullae lined by dense connective tissue. Bullae up to 1 cm in diameter were observed in a lung section from a pig that had a gross lesion in the right diaphragmatic lobe. All sterile soybean-treated pigs had foci of alveolar hemorrhage with intermixed macrophages and giant cells. Focal alveolar macrophage aggregates, multinucleated giant cells and interstitial granulomas were observed. Soybean particles were found within multinucleated giant cells in alveoli and in bronchioles. Soybean particles in bronchioles were surrounded by macrophages and a few neutrophils. Neutrophils and macrophages were located within cotyledon cells. The epithelial changes and inflammatory response in the bronchiolar wall were similar to those in soybean-treated pigs in previous

experiments. Although hemorrhagic bullous lesions were not observed in pigs receiving nonsterile soybean, other changes were similar in both groups of pigs.

No microscopic lesions were found in the trachea, tracheobronchial lymph node, caudal cervical lymph node, mesenteric lymph node, liver, or spleen of any pig. Cortical atrophy and interstitial fibrosis were found in grossly cystic kidneys.

Haemophilus parasuis was isolated from nasal secretions of the control pig that died spontaneously. Pathogenic bacteria were not isolated from nasal and tracheal secretions of other pigs. Four conventional pigs had low M. hyorhinis (1:4) complement-fixation antibody titers. Two of the 4 were control and 2 were soybean-treated pigs.

DISCUSSION

We were able to demonstrate that soybean particles could cause coughing, dyspnea, gross bullous and microscopic granulomatous lesions in swine. Therefore "plant-particle pneumonia" rather than "plant-particle associated pneumonia" is considered more appropriate terminology for this disease.

Immediate postinoculation coughing and dyspnea was observed in soybean-treated pigs. One control pig was found dead within 30 minutes of inoculation. Based on necropsy findings it probably had respiratory distress signs but the pig had not been closely observed following inoculation. The duration of dyspnea and coughing was longest in pigs that received 20 ml of 10% soybean, the most soybean particles given to any of the pigs. Bullous emphysema and multifocal hemorrhages were seen in one soybean-treated pig that had been given the high dose of soybean particles. Coughing is a reflex response which can be initiated by contact of particles with respiratory tract surfaces. When soybean particles occlude bronchioles, bronchospasms and cough responses are generated to dislodge the particles. Bullous emphysema and ruptured blood vessels may occur when air can be inspired but not expired as a result of bronchiolar obstruction and bronchospasm.

The causes of death for the 2 pigs that died spontaneously following inoculation were acute pulmonary and intrathoracic hemorrhage for the control pig and probably asphyxiation subsequent to bronchospasms for

the soybean-treated pig. Histologically, the soybean-treated pig had free plant particles in bronchioles and alveoli, but evidence of tissue injury was not found.

Pleural fibrosis observed grossly and microscopically in 2 conventional pigs was considered to be unrelated to soybean effects. Lesions of this type were possibly related to M. hyorhinis or H. parasuis infection.

Microscopic lesions occurred in both left and right lung lobes. Tilting the pig to the right after inoculation did not cause lesions to occur predominately on the right side.

Microscopic lesions in the absence of gross lesions were common in soybean-treated pigs in each experiment. Thirty-five of 55 soybean-treated pigs had only microscopic lesions. Apparently the limited, scattered foci of inflammation within lobules seen microscopically were insufficient for gross recognition.

Lesions seen in the experimental disease were not as extensive as those in the natural disease. Neutrophils, macrophages and multinucleated giant cells were admixed in bronchioles and alveoli of many pigs with natural plant-particle pneumonia (13). In the experimental disease, neutrophils were usually limited to soybean-obstructed bronchioles. Discrete interstitial granulomas and focal alveolar macrophage aggregates were seen in pigs with the experimental disease and pigs with the natural disease. However, the density of granulomas and macrophage aggregates in lung sections was greater in pigs with the natural disease.

The difference between lung lesions of experimentally-induced and naturally occurring plant-particle pneumonia could be due to duration of exposure. Lungs of naturally-affected self-fed pigs are probably exposed repeatedly to plant particles. Experimental pigs received only a single intratracheal dose of soybean particles. Since only one experimental control pig had a single microscopic focal granulomatous lung lesion, floor-feeding was not considered an important factor. In contrast to confinement-reared commercial pigs, our daily cleaning procedure would have reduced atmospheric dust particles, bacteria and toxic gases that may enhance plant-particle pneumonia lesion development.

Only a few experimental pigs had lesions suggestive of mycoplasmosis and these were mild. Lesions morphologically consistent with M. hyopneumoniae were seen in pigs with natural plant-particle pneumonia (1,13). These organisms become interposed between respiratory tract cilia. Decreased mucociliary transport is associated with mycoplasma-induced cilia damage (7). Defective pulmonary clearance may contribute to characteristics of naturally occurring plant-particle associated pneumonia.

Particle size may influence the characteristics of the lung lesions. Larger particles (over 265 μm) favored development of obstructive bronchiolitis. Smaller particles (under 105 μm) caused both bronchiolar and alveolar lesions.

Pigs receiving either 20 ml of nonsterile or ethylene oxide-sterilized 10% soybean had lesions that were quantitatively more extensive and qualitatively more severe than soybean-treated pigs which received

lesser doses in other experiments.

The similarity of clinical signs and lesions in pigs that received autoclaved, ethylene oxide-sterilized or nonsterile soybean would suggest that neither sterilization nor sterilization method had an important influence on characteristics of the experimental disease. However, sterilization would eliminate viable microorganism contamination.

For reproducing plant-particle pneumonia, a 20 ml dose of 15% soybean and possibly repeated doses should prove more effective than doses used in these experiments. Since neutrophils were not prevalent, bacterial interaction may be necessary for that characteristic of natural plant-particle pneumonia lesions. Neutrophils might have been more abundant if the pigs in these experiments had been sampled sooner after inoculation. Previous sequential studies of plant-particle pneumonia in laboratory animals have shown that acute bronchopneumonia is present at 24 hours postinoculation (6,12).

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Table I. Treatments, pigs per treatment and necropsy intervals

	Soybean Concentration (%)				Necropsy (PID[a])			
Exp	0[b]	2.5	5.0	10.0	7	14	21	Comments
A	1	4				5		Ten and 20 ml given 2 pigs each
B	8	8	8	8	16		16	Five or 10 ml given to 4 pigs per group. Two of each subgroup killed at 1 or 3 weeks.
C	2			6		8		Three pigs each given 10 ml of autoclaved or ethylene oxide sterilized soybean.
D	5			15		20		Five pigs each given 20 ml of saline containing size graded soybean particles.
E	3			6		9		Three pigs each given ethylene oxide sterilized or nonsterile soybean.
Total	19	12	8	35	16	42	16	

[a]Postinoculation day.

[b]Controls received an equivalent volume of sterile water or saline transtracheally.

Fig. 1: Alveoli and bronchioles of a saline treated control pig. No changes are present. HE stain. Bar = 100 µm.

Fig. 2: Multifocal lesions are in alveoli of a pig killed 7 days after receiving 10 ml of 5% autoclaved soybean suspension. Multinucleated giant cells and macrophages are in alveoli that are collapsed. No lesions are present in adjacent lobules. HE stain. Bar = 100 µm.

Fig. 3: Focal aggregate of epitheloid macrophages entirely fills an alveolus in a pig killed 21 days after receiving 5 ml of 2.5% autoclaved soybean suspension. HE stain. Bar = 20 µm.

Fig. 4: Foreign body type multinucleated giant cells are in alveoli of a pig killed 21 days after receiving 10 ml of 2.5% autoclaved soybean suspension. HE stain. Bar = 20 µm.

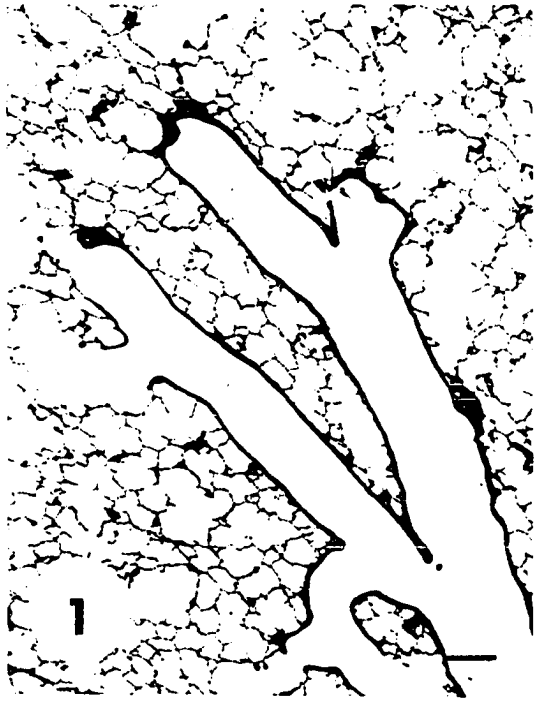
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Fig. 5: Focal interstitial granuloma with several Langhans type multinucleated giant cells enclosed by a zone of connective tissue fibroplasia. Lung from a pig killed 14 days after receiving 10 ml of 10% autoclaved soybean suspension. HE stain. Bar = 50 µm.

Fig. 6: Soybean obstructed bronchiole. Numerous neutrophils are within cotyledon cell cytoplasm and around the periphery of the plant particle. The bronchiolar epithelium is attenuated in the upper left quadrant of the mucosal surface. The bronchiolar lamina propria and peribronchiolar connective tissue is infiltrated by macrophages and neutrophils. Lung from a pig killed 7 days after receiving 10 ml of 10% autoclaved soybean suspension. HE stain. Bar = 50 µm.

Fig. 7: Soybean obstructed terminal bronchiole. Neutrophils and macrophages are within and around soybean cotyledon cells. There is mild peribronchiolar fibrosis and infiltration by macrophages and a few lymphocytes. Lung from a pig killed 14 days after receiving 10 ml of 10% of ethylene oxide-sterilized soybean suspension. HE stain. Bar = 50 µm.

Fig. 8: Soybean obstructed terminal bronchiole. Macrophages are along the periphery of the soybean particle. Only a few neutrophils are present. There is mild peribronchiolar fibrosis and macrophage infiltration. Lung from a pig killed 21 days after receiving 10 ml of 10% autoclaved soybean suspension. HE stain. Bar = 50 µm.


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PART III. SEQUENTIAL LESIONS OF EXPERIMENTAL SOYBEAN-INDUCED PLANT-PARTICLE PNEUMONIA OF SWINE

This manuscript will be submitted to Veterinary Pathology.

ABSTRACT

Clinical signs, gross and microscopic lung lesions were studied in sequentially necropsied conventional (35 pigs) and respiratory disease free (20) pigs receiving single or multiple 20 ml intratracheal doses of sterile soybean particles (15% by volume) or saline. Soybean particles consistently caused immediate postinoculation coughing and dyspnea in all 38 pigs receiving soybean. Intermittent coughing occurred up to 7 days postinoculation in some pigs. Three soybean-treated pigs died spontaneously after receiving one dose of soybean particles. Gross lesions including bullous emphysema, pulmonary hemorrhage, and diffuse yellow to gray lung mottling were found in 22 soybean-treated pigs. Microscopic lesions consistent with those of plant-particle pneumonia occurred in 35 of 38 soybean treated pigs and 2 of 17 controls. Conventional and respiratory disease free soybean-treated pigs receiving a single dose of soybean particles had acute bronchopneumonia when killed at 1 or 3 days postinoculation. By 7 days postinoculation, granulomatous inflammation was prominent. Interstitial granulomas formed as early as 7 days and were the principal lesions at 14 days postinoculation. Multiple dosed conventional pigs killed 1, 3, or 6 days after 3 doses of soybean particles had lesions that were simultaneously suppurative and granulomatous. Lesions of multiple dosed pigs are similar to lesions of natural plant-particle pneumonia. The similarity of bronchopneumonia and granulomatous lesions in respiratory disease free and conventional pigs

given sterile soybean suspensions indicates that bacterial interaction is not required for development of clinical signs and lesions of plant-particle pneumonia in swine.

INTRODUCTION

In a previous report, lesions of experimental soybean-induced pneumonia in swine (12) were not as severe or as extensive as those seen in lungs of swine with spontaneous plant-particle pneumonia (1,6,11, 14). Repeated and more concentrated doses of soybean particles coupled with earlier sampling after soybean inoculation were recommended as ways in which experimental reproduction of lesions of naturally occurring plant-particle pneumonia might be achieved (12).

Lung lesions observed in naturally occurring swine plant-particle pneumonia are similar to lesions of "lentil pulse pneumonia," a human disease caused by aspirated lentils, peas, beans or other vegetable materials (4,7,9). Lesions of lentil pulse pneumonia have been experimentally reproduced and studied sequentially in cats, rabbits and guinea pigs following intratracheal or intrapulmonary injections of broth from cooked peas, beans, or lentils. Purulent bronchopneumonia developed within 24 hours after broth administration and persisted approximately 10 days. At 10 days most lentils were walled off by giant cells, macrophages and lymphocytes. By 2 weeks postinoculation, lentils in granulomas had begun to disintegrate. At 2 to 3 weeks postinoculation, many granulomas had peripheral fibrosis without distinct encapsulation, while other granulomas were encapsulated and had central plant-laden macrophages. Fibrotic nodules and calcified nodules were still present 2 to 3 months after inoculation (7).

Pulmonary changes observed in sequentially killed mice and guinea pigs intranasally injected with saline suspended pine pollen also had an early inflammatory response dominated by neutrophils and macrophages. Neutrophil invasion of the pollen particles, suggestive of a chemotaxic response, was described. Granulomas were seen 2 months after exposure. Animals receiving endotracheal doses of pollen 12 months prior to intranasal exposure developed moderate to severe anaphylaxis and had intense chronic lung inflammation with numerous eosinophils (10).

This report describes the clinical signs, gross and histologic lesions that occurred in sequentially examined conventional and respiratory disease-free barrier-reared pigs receiving a single or multiple transtracheal dose of soybean particles.

MATERIALS AND METHODS

Three experiments were conducted to characterize clinical signs and sequential lung alterations that occurred in conventional pigs given single (exp A) and multiple transtracheal doses of soybean particles (exp C) and Laboratory Animal Resources respiratory disease-free (LAR) pigs (exp B) given a single transtracheal dose. In experiment A, 10 conventional pigs weighing 9.1 to 22.7 kg and 12 pigs weighing 24.5 to 39 kg were used. Characteristics of the LAR pigs have been previously reported (12). Twelve conventional pigs weighing 10 to 18 kg were used in experiment C. All experimental pigs were crossbreeds. They were housed and maintained as previously described (12). Representative microscopic lesions in formalin-fixed lung samples of conventional pigs, killed 14 days after receiving 20 ml of ethylene oxide-sterilized soybean (12), were also compared with lesions in experiment B (LAR) soybean-treated pigs killed 14 days postinoculation.

The soybean inoculum in all experiments was prepared as previously described (12). Each dose consisted of 20 ml of 15% ethylene oxide-sterilized soybean particles. Preinoculation and inoculation procedures have been previously reported (12).

In experiment A, one dose of soybean particles was given to 12 pigs and 20 ml of sterile saline was given to 10 control pigs. In experiment B, 16 pigs received a single dose of soybean and 4 pigs received a 20 ml dose of sterile saline. In experiment C, 10 pigs were

given a dose of soybean particles on experiment day 0, 3 and 10 while 3 control pigs received 20 ml doses of sterile saline on the same days. Soybean and saline treated pigs used in experiment A were killed 1, 3, and 7 days postinoculation. Soybean-treated pigs in experiment B were killed 1, 3, 7 and 14 days postinoculation. In experiment C, pigs treated with soybean were killed on experiment days 11, 13 and 16. Control pigs in experiment C were killed on experiment day 16.

Necropsy and histologic procedures were done as previously reported (12), except that selected soybean-treated and control pigs in experiments B and C had their lungs filled with modified Karnovsky's fixative (3). After lungs filled with modified Karnovsky's fixative were examined and photographed, they were individually wrapped with a fixative-soaked cloth towel, enclosed in a 4 l plastic bag and refrigerated until trimmed. When lungs fixed in modified Karnovsky's fixative were trimmed for light microscopy, a sample of adjacent tissue was removed and placed in fresh fixative and refrigerated. After light microscopic examination, selected lung samples adjacent to lung sections which had lesions as well as lung from control pigs were processed for semithin sectioning. Formalin-fixed lung from conventional pigs used in a previous experiment (12) which had been killed 14 days after receiving 20 ml of 10% ethylene oxide-sterilized soybean was also processed for semithin sectioning.

Semithin sections for light microscopy were obtained by preparing lung samples for electron microscopy. Lung samples fixed in modified

Karnovky's fixative were subdivided into 1 to 2 mm cubes and washed 3 times for 15 minutes in 0.1 M sodium cacodylate buffer with 1.7% sucrose, pH 7.2. They were postfixed 2 hours in 1% osmium tetroxide added to wash buffer. Samples were washed 3 times in distilled water and dehydrated in a graduated series of 50 to 100% alcohol and 2 changes of propylene oxide. The samples were embedded in epon-araldite plastic (2). After polymerization, semithin sections, 1 to 2 μm, were cut on an ultratome (LKB Ultrotome, model 8800, LKB Produkter-AB, Gaithersburg. Maryland) with glass knives. Semithin sections were stained with methylene blue-azure II-basic fuchsin stain (5).

RESULTS

In each experiment, dyspnea and coughing were observed in all soybean-treated pigs immediately after administration of soybean suspension. Coughing and dyspnea persisted for up to 3 hours post-inoculation. Thereafter, occasional coughing occurred in soybean-treated pigs when they were aroused in the process of obtaining rectal temperatures. No respiratory distress was observed in saline-treated pigs. Three pigs in the multiple dose experiment died on day 0 within 30 minutes after receiving soybean suspension.

The incidence of gross and microscopic lesions are presented in Table I for single-dosed pigs and Table II for multiple-dosed pigs. Gross lesions were limited to the respiratory tract and thorax and occurred in 22 of 38 soybean-treated pigs. No control pigs had gross lesions.

In experiment A, single and multiple variable-sized bullous lung lesions from 0.4 cm in diameter to 4.5 cm in diameter by 8 cm long were seen in 4 soybean-treated pigs killed one day after soybean treatment. Lesions occurred in all lung lobes, but more frequently in apical and caridac lobes. The lung parenchyma bordering a bulla capsule was dark red. Bullous cavities usually contained air and coagulated blood was adhered to the internal surface of bullae. Coagulated blood filled the entire cavity of a few bullae. Similar lesions were found in lungs of 2 soybean-treated pigs killed 3 days postinoculation (fig. 1,2).

In experiment B, one soybean-treated pig killed at 3 days post-inoculation had a 1 cm bullous focus in the intermediate lobe and multiple 1 to 2 cm reddened subpleural foci in the left apical, both cardiac and both diaphragmatic lobes. Two other soybean-treated pigs killed at 3 days postinoculation had 1 to 2 cm red subpleural foci in their cardiac or diaphragmatic lobes. Diffuse yellow to gray mottling of cardiac, intermediate and diaphragmatic lobe parenchyma was observed in 3 experiment B soybean-treated pigs killed 7 days postinoculation.

Gross lesions were found in all soybean-treated pigs used in the multiple dose experiment. No gross lesions were found in any control pigs (Table II). Diffuse pulmonary hemorrhage, pulmonary bullae, ruptured pulmonary pleura and intrathoracic hemorrhage were observed in 3 pigs that died after soybean-treatment. Diffuse yellow to gray mottling was seen in lungs of other multiple dose pigs killed 1, 3, or 6 days after the last soybean dose. Multifocal bullae with fibrous blood-coated capsules were found in cardiac and diaphragmatic lobes.

Unilateral or bilateral atrophy of ventral scrolls of ventral turbinates was observed in 2 control and 4 soybean-treated conventional pigs used in experiment A. Similar turbinate changes were observed in 2 control and 4 soybean-treated conventional pigs used in the multiple dose experiment.

Microscopic lung lesions were found in all soybean-treated pigs used in each experiment, one control pig in experiment A and one control pig in the multiple dose experiment. Control pigs used in experiment B

and other controls without lung lesions had lung structure similar to the representative example shown in figure 3. Soybean-associated lesions in experiment A and B pigs were similar at 1, 3, and 7 days postinoculation. Soybean induced lesions in a conventional pig killed 14 days postinoculation in another experiment (12) were also similar to lesions at 14 days in experiment B pigs.

At day 1 postinoculation, neutrophil exudation into alveoli and bronchioles was the predominant change (fig. 4). Foci of alveolar interstitial edema with neutrophil infiltration were seen in most lobules. Alveolar epithelial cells frequently had vacuolated cytoplasm. Dilated lymphatics containing macrophages and lymphocytes were occasionally seen alongside bronchioles. Plant particles 10 to 70 μm in size were seen free in alveoli surrounded by neutrophils. Similar inflammatory responses to larger (50 to 250 μm) plant particles were seen in bronchioles. Soybean cotyledon cells were often infiltrated by neutrophils. All animals with gross bullous lesions also had free erythrocytes, neutrophils and plant particles admixed in the alveoli and bronchioles. Bullous lesions were usually subpleural, between lobules or within individual lobules. Either partially or completely occluded terminal bronchioles were frequently observed at the entrance to emphysematous lobules. Blood-filled subpleural hematomas were also observed.

Soybean-treated pigs killed at 3 days postinoculation had neutrophils admixed with macrophages in alveoli and bronchioles. Erythrocyte aggregates were observed in alveoli of animals with hemorrhagic cysts. Giant cells were seen as early as 3 days after

a single dose of soybean particles (fig. 5). Plant particles in alveoli were usually surrounded by neutrophils and macrophages, but were also seen occasionally in giant cells (fig. 6). The alveolar wall was lined by vacuolated epithelial cells. Neutrophils and macrophages were observed in edematous alveolar interstitial connective tissue.

Macrophage aggregates and multinucleated giant cells were abundant in alveoli by 7 days after a single dose of soybean. Alveolar epithelial hyperplasia and perivascular fibroplasia were also detectable (fig. 7,8). In some lung sections, contracted alveoli were closely apposed to multinucleated giant cells. Soybean cotyledon fragments in obstructed bronchioles were usually surrounded by macrophages and neutrophils. Neutrophils were most abundant when cotyledon cells still contained cytoplasm. Bronchiolar epithelium was attenuated or eroded. Neutrophils and macrophages were in the lamina propria (fig. 9). Giant cells and macrophages were prominent in a few soybean-obstructed bronchioles.

Granulomas were seen in alveolar interstitium as early as 7 days postinoculation. By 14 days postinoculation, multifocal granulomas were the principal lesions. Granulomas consisted of either single or multiple central multinucleated giant cells surrounded by a connective tissue zone. Plant particles were often in the multinucleated giant cells. Alveolar epithelium of adjacent alveoli surrounded the connective tissue zone (fig. 10). Capillaries were also seen in the connective tissue zone near the epithelial surface of adjacent alveoli. Foci of perivascular fibrosis and alveolar interstitial fibrosis

could be identified in many lung sections. Alveolar spaces contained few inflammatory cells other than occasional macrophage aggregates and multinucleated giant cells.

Hyperplastic alveolar epithelium was still present in most alveoli at 14 days postinoculation. Bronchioles were either occluded by giant cells and macrophages or contained soybean cotyledons enclosed by the bronchiolar wall connective tissue.

Granulomatous lesions in the lung of a conventional soybean-treated pig killed at 14 days postinoculation were similar to lesions in experiment B pigs killed at the same interval after receiving a single dose of soybean suspension.

Multiple dosed pigs killed 1, 3, or 6 days after receiving their last dose of soybean had diffuse acute and chronic lesions. Neutrophils were most abundant in pigs killed 1 or 3 days after the last dose was given. Alveolar walls were infiltrated by neutrophils, macrophages, and lymphocytes. Interstitial fibroplasia was frequently observed. Alveolar spaces contained macrophage aggregates and multinucleated giant cells (fig. 11). Soybean particles were free in alveoli and within macrophages and multinucleated giant cells. Fewer inflammatory cells were seen in alveoli at 6 days after the last soybean dose. Type II pneumocyte hyperplasia was clearly distinguishable in semithin lung sections of soybean-treated pigs (fig. 12). Various stages of granuloma formation were found. In one semithin section, multinucleated giant cells adhered to a segment of denuded fibroplastic alveolar wall, while type I and type II

pneumocytes lined the remainder of the alveolar wall (fig 13). Complete circumferential alveolar interstitial fibrosis was seen in most granulomas. The amount of connective tissue between the epithelium of adjacent alveoli and the multinucleated giant cells varied. Concentrically arranged fibroblasts in 3 to 4 layers were seen around some multinucleated giant cells. A few macrophages and lymphocytes were located within the connective tissue of granulomas.

Bronchiolar lesions were also variable. Neutrophils were seen in protein bodies of soybean cotyledons in bronchioles with little epithelial damage (fig 14). Bronchioles with denuded epithelium and fibrosed lamina propria often had macrophages and multinucleated giant cells in their lumen. Plant particles were not clearly recognizable as soybean in fibrosed occluded bronchioles. Frequently the bronchiolar smooth muscle layer was the only recognizable bronchiolar feature.

One single-dosed control pig in experiment A and one multiple dosed control pig had 1 to 3 foci of alveolar multinucleated giant cells or macrophage aggregates in 2 lung lobes.

Parabronchiolar and perivascular lymphoid hyperplasia was observed in 4 soybean-treated and 5 control pigs used in experiment A and 1 soybean-treated and 3 control pigs used in the multiple dose experiment. Pigs having only pulmonary lymphoid hyperplasia were not included in Table I and II incidence data.

No microscopic lesions were found in trachea, tracheobronchial lymph node, caudal cervical lymph node, mesenteric lymph node, liver,

spleen, or kidney sections from any pig in experiments A, B, or C.

Complement-fixation antibody titers to Mycoplasma hyopneumoniae of 1:4 to 1:32 were detected in serum samples from 10 soybean-treated and 7 control conventional pigs that received either single or multiple doses. Complement-fixation antibody titers to Mycoplasma hyorhinis of 1:8 to 1:32 were determined for sera from 3 conventional pigs that received either soybean or saline. Pasteurella multocida was recovered from the nasal cavities of 3 experiment A and 1 experiment C pigs. Bordetella bronchiseptica was recovered from the nasal cavities of 5 experiment A and 3 experiment C pigs.

DISCUSSION

Single and multiple 20 ml doses of 15% soybean particles suspended in saline consistently caused immediate postinoculation dyspnea and coughing in all 38 soybean-treated pigs used in these studies. Coughing occurred in soybean-treated pigs until the 7th postinoculation day in single dosed pigs and until the 6th postinoculation day after the last dose in multiple dosed pigs. It is likely that the soybean particles irritated the respiratory tract for an extended period of time which resulted in the coughing. Also, the finding of gross lesions in 22 of 38 soybean-treated pigs suggests prolonged irritation probably occurred. Coughing did not extend beyond the first day in soybean-treated pigs studied previously. However, in one group of pigs receiving a high dose of soybean they did cough for several hours after inoculation compared to minimal coughing in pigs receiving lower doses (12).

Gross lesions were either hemorrhagic and bullous or diffuse yellow to gray mottling. The hemorrhagic and bullous lesions probably resulted from bronchiolar obstruction whereas alveolar consolidation due to inflammation and atelectasis was the apparent cause of the mottling. The latter gross change has not been observed previously in experimental plant particle pneumonia of swine. Experiment A and B pigs had both types of lesions, whereas, only mottling occurred in experiment C pigs.

The inflammatory response to soybean particles proceeds from acute to chronic. At 1 or 3 days postinoculation, neutrophils were abundant in alveoli and bronchioles of all soybean-treated pigs given a single

dose. None of the control pigs examined at 1 or 3 days postinoculation had suppurative bronchopneumonia. As in the natural disease (11), neutrophils had an affinity for soybean cotyledon cell cytoplasm. Bronchiolar erosion without fibrosis occurred during the acute stages, from 1 to 7 days postinoculation. Although the suppurative response did overlap the granulomatous response, the reaction at 7 days was primarily granulomatous. Multinucleated giant cells can form early in response to soybean particles. They were first seen at 3 days in a few single dosed pigs. Acute interstitial inflammation occurs along with exudative changes. Interstitial inflammation probably occurs as a result of the effects of chemical mediators of inflammation rather than as a direct effect induced by plant particles.

Bordetella bronchiseptica and P. multocida were recovered from the nasal cavities of experiment A and C pigs but not from their tracheas. Mycoplasma-associated pulmonary lymphoid hyperplasia and mycoplasma CF antibody titers were also demonstrated. It is unlikely that these organisms were primarily involved in the suppurative response in alveoli and bronchioles since neutrophilic responses were also predominant in respiratory disease-free pigs killed at 1 or 3 days postinoculation and experiment A and C control pigs did not have suppurative bronchopneumonia.

When multinucleated giant cells were present in alveolar spaces the alveolar wall was occasionally closely apposed to them. This may represent alveolar collapse subsequent to loss of pulmonary surfactant (13), interaction of physical factors such as small alveolus size and increased multinucleated giant cell size, or some mechanism that

facilitates isolation of phagocytized foreign bodies. The latter possibility is suggested since fibrous connective tissue was seen at the periphery of some macrophages in lung sections of pigs killed at 7 and 14 days after a single dose.

Bronchiolar changes also became chronic with time. Macrophages, multinucleated giant cells and neutrophils were present in and around obstructing soybean particles and in the eroded bronchiolar wall by 7 days. Fibrosis of the bronchiolar wall was prominent at 14 days postinoculation.

The finding of acute and chronic lesions in the same lung sections of multiple-dosed animals suggests that repeated exposure may be an important contributory factor to characteristics of the natural disease. Although the lungs of single-dosed sequentially-killed pigs provided a better representation of the progression of lesion from acute to chronic, multiple dosed pigs had lesions most similar to natural plant-particle pneumonia (1,6,11,14).

Three spontaneous deaths which occurred shortly after inoculation of soybean suspension were attributed to lung rupture and intrathoracic hemorrhage. This probably represents a fatal endpont of acute bullous emphysema which develops when bronchioles are obstructed by the particles.

Microscopic lung lesions found in 2 control pigs were considered incidental since only a few multinucleated giant cells and macrophages were observed.

Microscopic lung lesions observed in single-dosed pigs are similar

to sequential lesions described in experimental lentil pulse pneumonia (7) and experimental pine pollen granuloma (10) in laboratory animals.

We conclude that acute bronchopneumonia can be induced by soybean particles without bacterial interaction. Plant-particle pneumonia lesions that are acute and chronic can coexist as a result of multiple dose lung exposures in swine. Multiple-dose effects are more representative of natural plant-particle pneumonia in swine than are single-dose effects.

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Table I. Incidence of gross and microscopic lesions of plant particle pneumonia in sequentially necropsied pigs receiving a single intratracheal dose of 15% soybean particles in 20 ml sterile saline

		Gross lesions		Microscopic lesions[a]	
Exp	Postinoculation Day	Soybean	Saline	Soybean	Saline
A	1	4/4[b]	0/3	4/4	1/3[c]
	3	2/4	0/3	4/4	0/3
	7	0/4	0/4	4/4	0/3
B	1	0/4	0/2	4/4	0/2
	3	3/4		4/4	
	7	3/4		4/4	
	14	0/4	0/2	4/4	0/2

[a]Data does not include pigs with parabronchiolar and perivascular lymphoid hyperplasia.

[b]Pigs with lesions/pigs examined.

[c]Focus of macrophages and multinucleated giant cells in alveoli in one or more lung sections.

Table II. Incidence of gross and microscopic lesions of plant particle pneumonia in sequentially necropsied pigs receiving multiple intratracheal doses of 15% soybean particles in 20 ml sterile saline on day 0, 3 and 10. (Experiment C)

	Gross lesions		Microscopic lesions	
Necropsy Day[a]	Soybean	Saline	Soybean	Saline
0[b]	3/3[c]		2/2[d]	
11	2/2		2/2	
13	2/2		2/2	
16	3/3	0/3	2/2	1/3[e]

[a]Necropsy day is the experiment day when pigs were killed.

[b]Three pigs died within 30 minutes after inoculation.

[c]Pigs with lesions/pigs examined.

[d]One lung not examined.

[e]Spontaneous focus of intraalveolar giant cells observed in 2 lung sections from one pig.

Fig. 1: A bullous lesion (arrow) is in the right diaphragmatic lobe of a pig killed 1 day after receiving 20 ml of ethylene oxide-sterilized 15% soybean suspension. The dark areas are coagulated blood that coated the bullous cavity.

Fig. 2: A bullous and hemorrhagic focus (arrow) is in lung from a pig killed one day after receiving 20 ml of ethylene oxide-sterilized 15% soybean suspension. A smaller lesion is cranial to that focus. The dorsal portion of the left cardiac lobe also has a bullous and hemorrhagic focal lesion.

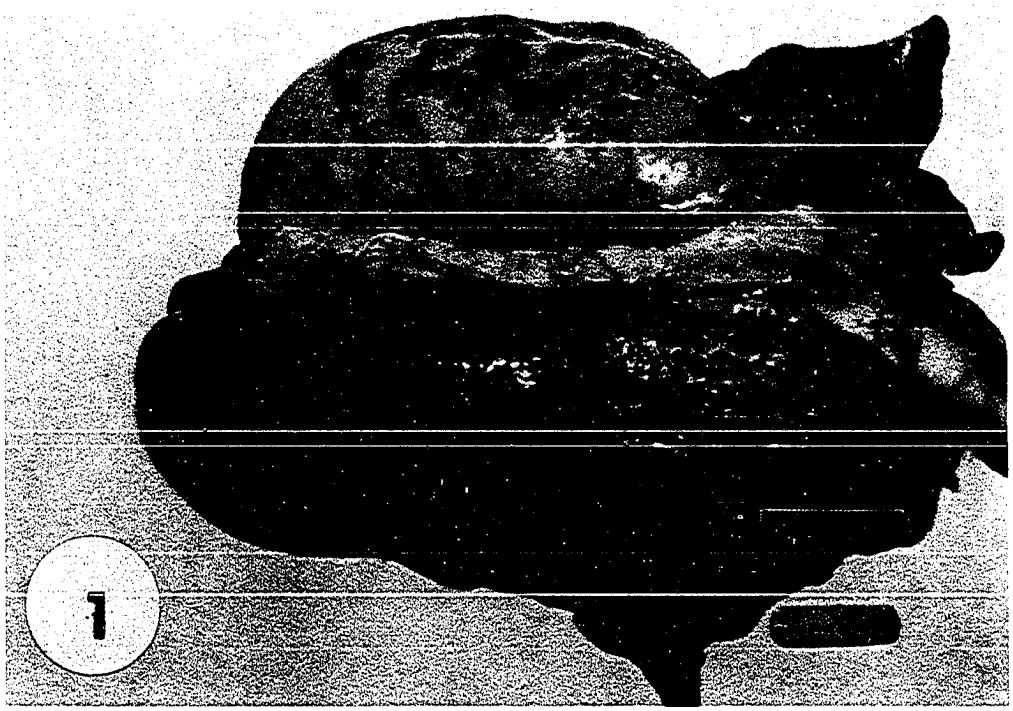
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Fig. 3: Note the lung structure of a barrier-reared respiratory disease free pig killed one day after receiving 20 ml sterile saline transtracheally. A terminal bronchiole and adjacent alveoli are shown. No structural alterations are present. HE stain. Bar = 100 µm.

Fig. 4: Suppurative bronchopneumonia is apparent in lung from a barrier-reared respiratory disease-free pig killed 1 day after receiving 20 ml ethylene oxide-sterilized 15% soybean suspension. Neutrophils have surrounded a soybean particle in an alveolus. A few macrophages are in the alveoli. The connective tissue of alveolar septa is edematous and infiltrated by neutrophils and macrophages. HE stain. Bar = 20 µm.

Fig. 5: Giant cells in lung lesions of a conventional pig killed 3 days after receiving 20 ml of ethylene oxide-sterilized 15% soybean suspension. Multinucleated giant cells are in alveoli near upper left corner of the photomicrograph. This pig had gross focal pulmonary hematomas. Several alveoli contain free erythrocytes among macrophages and neutrophils. A fragment of soybean coytledon is in the bronchiolar lumen. HE stain. Bar = 100 µm.

Fig. 6: Soybean cotyledon cells are within the cytoplasm of a multinucleated giant cell. The alveolar epithelium is closely apposed to the periphery of the multinucleated giant cell. Adjacent alveoli which are partially collapsed contain free erythrocytes and a few macrophages. HE stain. Bar = 50 µm.


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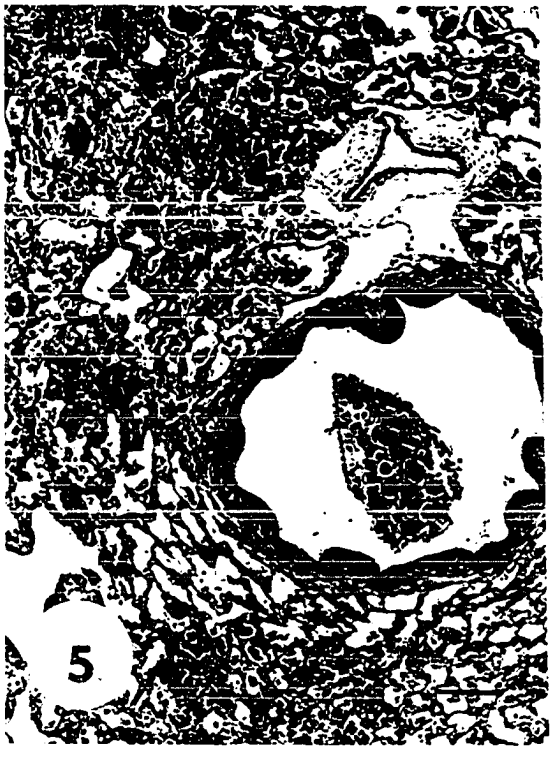
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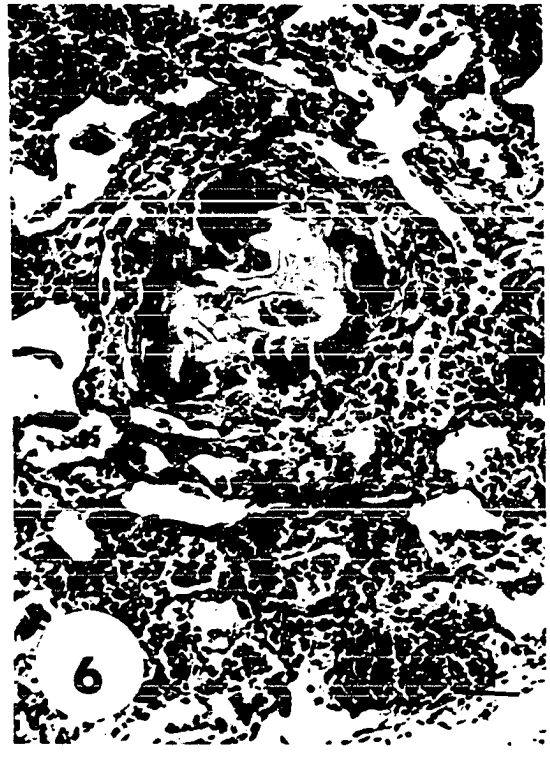
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Fig. 7: Multinucleated giant cell and macrophage aggregates are in alveoli of lung from a barrier-reared respiratory disease free pig killed 7 days after receiving 20 ml ethylene oxide-sterilized 15% soybean suspension. The alveolar epithelium is hyperplastic. HE stain. Bar = 50 µm.

Fig. 8: Another section from the pig described in figure 7 shows Langhans giant cells in alveoli along with smaller macrophages. There is a marked interstitial fibroplasia and alveolar epithelial hyperplasia. HE stain. Bar = 50 µm.

Fig. 9: Soybean cotyledon obstructed bronchiole. The cotyledon cell cytoplasm is infiltrated by neutrophils. Neutrophils are admixed with macrophages in the alveoli and bronchiolar wall connective tissue. Lung from a conventional pig killed 7 days after receiving 20 ml of ethylene oxide-sterilized soybean suspension. HE stain. Bar = 50 µm.

Fig. 10: Interstitial granuloma in a conventional pig killed 14 days after receiving 20 ml of 10% ethylene oxide-sterilized soybean suspension. Multinucleated giant cells are surrounded by a connective tissue zone. Epithelium of adjacent alveoli is intact. 1-2 m epon-araldite section. Methylene blue-azure II-basic fuchsin stain. Bar = 50 µm.


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Fig. 11: Simultaneous acute and chronic inflammatory response in a multiple dosed pig killed 3 days after receiving the last of three 20 ml doses of ethylene oxide-sterilized 15% soybean suspension over a 10 day period. The alterations shown are interstitial fibroplasia, multinucleated giant cells, and epithelial hyperplasia. Neutrophils in the alveoli represent the acute response. 1-2 μm epon-araldite section. Methylene blue-azure II-basic fuchsin stain. Bar = 50 μm.

Fig. 12: Marked Type II pneumocyte hyperplasia in a multiple dosed pig killed 3 days after receiving the last of three 20 ml doses of ethylene oxide-sterilized 15% soybean suspension over a 10 day period. Surfactant granules are abundant in the apical cytoplasm of Type II pneumocytes. Soybean particles are in the cytoplasm of a multinucleated giant cell. Macrophages and desquamated epithelial cells are in the alveoli. 1-2 μm epon-araldite section. Methylene blue-azure II-basic fuchsin stain. Bar = 20 μm.

Fig. 13: Segmental adherence of multinucleated giant cells to alveolar wall is shown in this photomicrograph. The alveolar epithelium is closely apposed to giant cell surfaces. The interstitium beneath the adhered alveolar epithelium is fibroplastic. The unadhered segment of the alveolus is lined by both Type I and Type II pneumocytes. A soybean particle (arrow) is within the mass of giant cells. Type II pneumocyte hyperplasia is present in a few alveoli. Lung from a multiple-dosed pig killed 6 days after receiving three the last of 20 ml doses of ethylene oxide-sterilized 15% soybean suspension. 1-2 μm epon-araldite section. Methylene blue-azure II-basic fuchsin stain. Bar = 20 μm.

Fig. 14: Soybean cotyledon in a bronchile. Protein bodies (PB) in the cotyledon cell have been infiltrated by neutrophils (arrow). Lung from a pig killed 3 days after receiving the last of three 20 ml doses of ethylene oxide-sterilized 15% soybean suspension. 1-2 μm epon-araldite section. Methylene blue-azure II-basic fuchsin stain. Bar = 20 μm.


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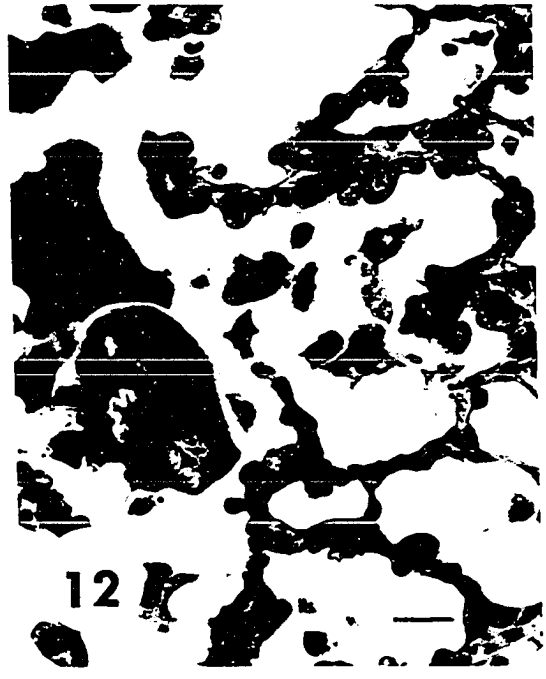
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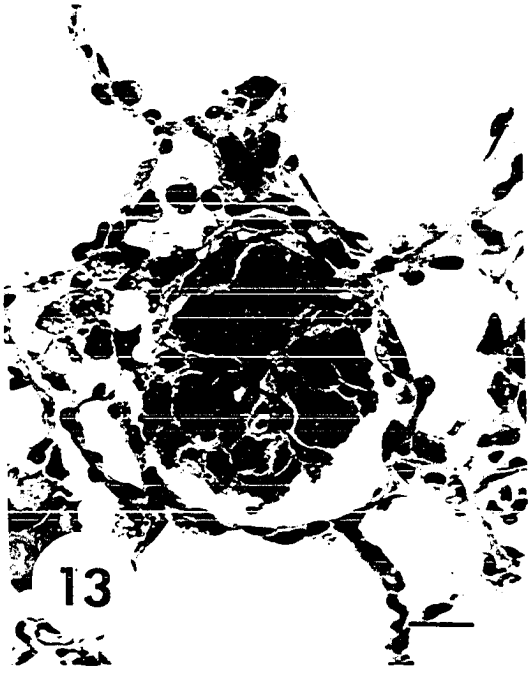
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PART IV. PATHOGENESIS OF INTERSTITIAL GRANULOMA FORMATION IN EXPERIMENTAL PLANT-PARTICLE PNEUMONIA OF SWINE. AN ULTRASTRUCTURAL STUDY

This manuscript will be submitted to Veterinary Pathology.

ABSTRACT

Transmission electron microscopy was used to study lung samples from conventional pigs that had been sequentially killed after receiving single or multiple doses of soybean particles or saline. All alveolar lesions that occurred in a single or multiple-dosed pig were not morphologically identical, however the majority of lesions usually reflected the predominant stage of lesion development. Between 1 and 3 days neutrophils were abundant especially within and around soybean particles. Macrophages increased after 3 days and by 6 or 7 days postinoculation macrophages were more abundant than neutrophils. At that time macrophage aggregation and fusion to form multinucleated giant cells was also prominent. In single-dosed pigs, early alveolar lesions included interstitial edema along with neutrophil and macrophage infiltration. Type II pneumocyte hyperplasia was frequently seen by 7 days postinoculation. Multinucleated giant cell and granuloma formation were the principal features observed after 7 days in single-dosed pigs.

Interstitial granulomas with or without plant particles form when multinucleated giant cells are entrapped in alveoli following epithelial and stromal damage. Tissue-damaging and fibrogenic factors, which may be secreted by multinucleated giant cells, could be responsible for interstitial granuloma formation.

INTRODUCTION

Pulmonary interstitial granulomas with and without internalized plant particles have been observed in naturally occurring (6,9,23,29) and experimental soybean-induced (24,25) plant-particle pneumonia in swine, and in plant-particle pneumonias in other species (7,11,22,28). Phagocytized plant particles in pulmonary interstitial granulomas frequently exceed 20 μm (6,24).

Translocation of phagocytized particles from the alveolar space into alveolar interstitium is not clearly understood. Free particles under 0.02 μm penetrate the intact alveolar epithelial barrier and are cleared by lymphatics (12,18). Some researchers believe that phagocytized particles cannot be translocated through the alveolar epithelial barrier to the interstitium (3,20,21). Kaolinite pneumoconiosis lesions were used to illustrate that dust particles could damage alveolar epithelium. Kaolinite particles were engulfed by macrophages which then adhered to the damaged surface. Macrophages later became internalized in the alveolar interstitium when new alveolar epithelium proliferated over the adhered macrophage aggregate (21). Dust particles over 10 μm were thought to remain in the alveoli until fragmented into smaller particles which could be phagocytized, and concurrently cause chronic interstitial inflammation which leads to alveolar obliteration (21).

Plant-particle pneumonia induced by soybean (25) and other plant materials (1,22) in sequentially killed animals is characterized by

early suppurative bronchopneumonia that gradually becomes granulomatous by about one week. At that time, both multinucleated giant cells and macrophages are prominent. After 2 weeks, predominant lesions are interstitial granulomas surrounded by variable amounts of peripheral fibrosis with or without plant particles in central macrophages. Experimental soybean-induced pulmonary granulomas in sequentially killed pigs consisted of multinucleated giant cells surrounded by a proliferative connective tissue zone infiltrated by macrophages and occasionally lymphocytes. The limiting structure around some granulomas was alveolar epithelium lining adjacent alveoli (25). However, some alveoli observed in semithin lung sections contained giant cells partially adhered to a connective tissue zone that was continuous with the alveolar interstitium. The unadhered portion of the alveolar wall was frequently lined by hyperplastic type II pneumocytes.

We propose that multinucleated giant cells, with or without observable engulfed foreign material, are responsible for alveolar epithelial damage which leads to alveolar obliteration and interstitial granuloma formation. Ultrastructural evidence is presented that illustrates the sequence of swine pulmonary reactions to soybean which ultimately leads to development of pulmonary interstitial granulomas.

MATERIALS AND METHODS

Epon-araldite embedded swine lung samples were used to characterize the ultrastructure of acute and chronic lesions induced by single and multiple doses of soybean particles. Lung from saline-treated control pigs was also examined. The epon-araldite embedded swine lung samples sectioned for transmission electron microscopy (TEM) were those semithin-sectioned for a previous light microscopic investigation of soybean-induced lesions in sequentially killed pigs (25). Ultrathin sections, 600-900 angstroms, were cut with a diamond knife-equipped ultratome (LKB Ultrotome, model 8800, LKB Produkter-AB, Gaithersburg, Maryland). Sections were mounted on copper grids, stained for 10 minutes with 2% uranyl acetate in methanol and 10 minutes in Reynold's lead citrate (17). TEM and photography was done on a Hitachi HS-9 electron microscope (Hitachi Perkin-Elmer, Chicago, Illinois) at 75 kilovolts.

RESULTS

The sequence of alveolar structural changes induced by soybean particles is outlined diagrammatically in figure 1. Control pigs killed at 1 day or 14 days after receiving saline transtracheally have alveoli free of epithelial or interstitial injury (fig 2,3). A slight increase in pinocytotic vesicles in type I pneumocytes and endothelium was found in control pigs killed 1 day postinoculation compared to control pigs killed 14 days postinoculation. Alveolar lesions that occurred in a single- or multiple-dosed soybean-treated pig did not develop at the same rate, however, the majority of lesions seen at one time reflected the predominant stage of lesion development.

Soybean-treated pigs killed 1 day postinoculation had free alveolar soybean particles or particles surrounded and invaded by neutrophils (fig 4). Phagolysosomes containing plant fragments were located in the cytoplasm of many neutrophils (fig 5). Neutrophils with and without phagocytized plant material were observed in alveoli that did not contain free plant particles. The alveolar wall was unaltered in some sections although neutrophils and plant materials were present. In most alveoli, epithelial and endothelial cells had many pinocytotic vesicles along their cell membranes. Interstitial edema, infiltration by neutrophils and a few macrophages were observed in some alveolar septa.

At 3 days postinoculation, numerous macrophages and neutrophils were located within soybean cotyledon fragments, alveoli, and interstitium. Phagocytic activity was directed at clearing the cotyledon

cell cytoplasm which consists primarily of protein bodies (fig 6). Alveolar epithelium was essentially unchanged from its appearance in pigs killed one day after inoculation.

At 6 to 7 days postinoculation many soybean cotyledon cells contained macrophages in their cytoplasmic compartments but no cytoplasm (fig 7). Some macrophages contained cell wall material. Macrophages with extensive cytoplasmic processes were also loosely aggregated around soybean particles (fig 8). Some macrophages were in contact with the alveolar epithelium but plasma membranes of both were intact. In some alveoli, macrophage aggregates were characterized by interlocking cytoplasmic processes and closely apposed smooth-contoured plasma membranes (fig 8,9). Sites of macrophage plasma membrane fusion could be identified at adjacent surfaces of aggregated macrophages (fig 10).

Giant cells were seen as early as 3 days postinoculation but were more abundant at 7 and 14 days postinoculation (fig 11). Langhans giant cells contained peripheral nuclei and abundant organelles, especially rough endoplasmic reticulum, lysosomes, and mitochondria. Foreign body giant cells had nuclei distributed througout the cytoplasm with fused plasma membranes and plasma membrane fragments between adjacent nuclei. In many alveoli, the epithelium adjacent to macrophage aggregates and giant cells was intact even though it was closely apposed to macrophage and giant cell surfaces. However, some alveoli had focal epithelial loss and giant cell cytoplasmic processes that were directly adjacent to or penetrated epithelial basement membranes. Frequently hyperplastic type II pneumocytes were located at the margins of the

denuded focus. Macrophages and a few fibroblasts were frequent in the interstitium beneath denuded foci. Few collagen fibers were detected in foci with few fibroblasts (fig 12). However, in more chronic lesions, seen as early as 7 days, there was extensive interstitial fibroplasia and large numbers of collagen fibers (fig 13,14).

By 14 days postinoculation interstitial collagen fibers were more abundant and organized than at 7 days postinoculation (fig 15). Macrophages and occasionally lymphocytes could be found among fibroblasts and collagen fibers around giant cells. Connective tissue proliferation involved all or part of the alveolar surface around giant cells. Plant particles or their remnants were observed in giant cells and macrophages.

The epithelium of alveoli adjacent to those containing entrapped giant cells was intact at both 7 and 14 days postinoculation. Type II pneumocyte hyperplasia and interstitial infiltrates of macrophages and lymphocytes were observed in alveoli that lacked alveolar exudate or plant materials at 7 and 14 days (fig 16).

Granulomas were also observed in terminal bronchioles. They had morphological features similar to granulomas in alveoli; however, larger soybean particles were usually present. Early bronchiolar exudative reactions to soybean particles were similar to alveolar reactions. Bronchiolar epithelium in contact with plant particles or giant cells at 7 or 14 days postinoculation was attenuated, denuded or replaced by connective tissue.

DISCUSSION

Characteristics of the inflammatory cell response to soybean particles in swine lung sections examined ultrastructurally agree with those described by light microscopy (24,25). The sequence of events we describe emphasizes a crucial role for macrophages and giant cells in the formation of interstitial granulomas. Macrophage aggregation and fusion can occur in the alveolar space. Contact between multinucleated giant cells and alveolar interstitium can occur if the alveolar epithelium is lost. Such contact is necessary for the fibroplastic response to occur that ultimately leads to interstitial granuloma formation. The alveolus remains patent until the alveolar wall is completely adhered to the multinucleated giant cell by connective tissue.

Macrophages and multinucleated giant cells are known to secrete proteases, collagenase, and elastase which can damage alveolar epithelium and interstitial connective tissue (4,14,26,27). These and perhaps other unidentified substances elaborated by the activated macrophages reacting to the soybean particle damage the alveolar epithelium. Macrophages also secrete a factor which stimulates fibrogenesis (4,13). Consequently, viable multinucleated giant cells and macrophages, which were once free in the alveolar space, can become entrapped in the interstitium through their action on the alveolar epithelium and underlying stroma.

Soybean-induced granuloma formation differs from granuloma development following phagocyte death. In the latter process, phagocytes are

killed by phagocytized material. The cell undergoes lysis releasing tissue-damaging and fibrogenic factors. Granuloma formation in asbestosis and silicosis (2,18) occurs by this process. Phagocyte death was not observed in soybean-induced granulomas. Perhaps the more destructive lung diseases induced by asbestos or silica compared to plant particles may relate to this basic difference in pathogeneic mechanisms.

Biologically active substances from soybean protein bodies probably attract inflammatory cells directly or may cause leukocyte-chemotaxis by activating the host humoral amplification system (8,10). Our description of macrophage aggregation and fusion to form multinucleated giant cells is similar to previous reports (1,15,19). Soybean protein bodies contain lectins (16). These materials can directly stimulate macrophage fusion (5) or stimulate lymphocytes to release lymphokines that cause cell fusion (19).

The inflammatory response to soybean particles is directed at disintegrating or isolating them. If the phagocytized particles are not cleared from the alveolus, an interstitial granuloma may result. However, the lesion remains strictly localized since the fibrogenic response at 14 days spared adjacent alveolar surfaces. This finding suggests that the interstitial granuloma, although locally injurious, is a closely controlled repair response designed to preserve as many functional alveoli as possible.

Soybean-induced pulmonary interstitial granuloma in swine may be

a useful animal model for evaluating pulmonary structural responses to foreign organic particles. Since soybeans contain lectins and other biologically active components (16) further studies are indicated to evaluate their effects on the immune system and inflammatory responses in swine lung.

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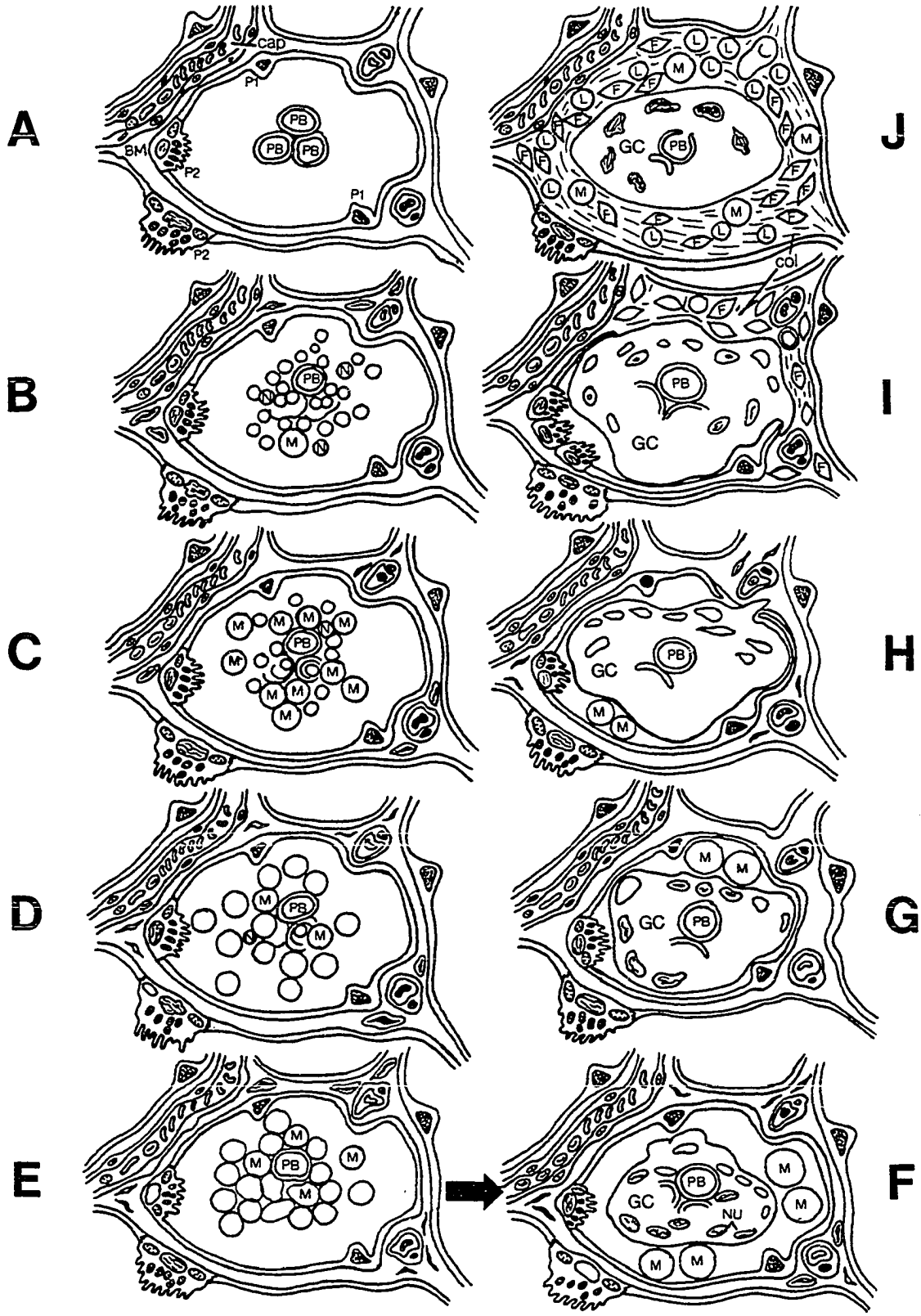
Fig. 1: Pathogenesis of soybean induced granuloma formation in swine lung. The sequential inflammatory responses and alveolar structural alterations that result in translocation of soybean particles into the interstitium are illustrated. (A) Undamaged alveolus with free soybean particle. (B) Neutrophil predominant infiltrate. (C) Neutrophil and macrophage infiltration. (D) Macrophage predominant infiltrate. (E) Macrophage aggregation and fusion. (F) Multinucleated giant cell with phagocytized soybean particle. (G) Alveolar collapse. (H) Alveolar epithelial damage. (I) Interstitial fibroplasia. (J) Interstitial fibrosis and multinucleated giant cell entrapment. Abbreviations: BM = basement membrane and interstitial matrix, cap = capillary, col = collagen fibers, F = fibroblast, GC = multinucleated giant cell, L = lymphocyte, M = Macrophage, N = neutrophil, NU = nucleus, P1 = type I pneumocyte, P2 = type II pneumocyte, PB = soybean protein body.


A
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NU

Fig. 2: Alveolar wall from a barrier-reared respiratory disease-free control pig killed 1 day after receiving 20 ml sterile saline transtracheally. A type 1 pneumocyte (P1) is shown. Notice surfactant layer on lumenal surface. The basement membrane (bm) is part of the interstitium (in). Collagen fibers are in the interstitium around the capillary endothelium (en). Note pinocytotic vesicles in epithelial and endothelial cells (arrows). Uranyl acetate and lead citrate stain. Bar = 1 μm.

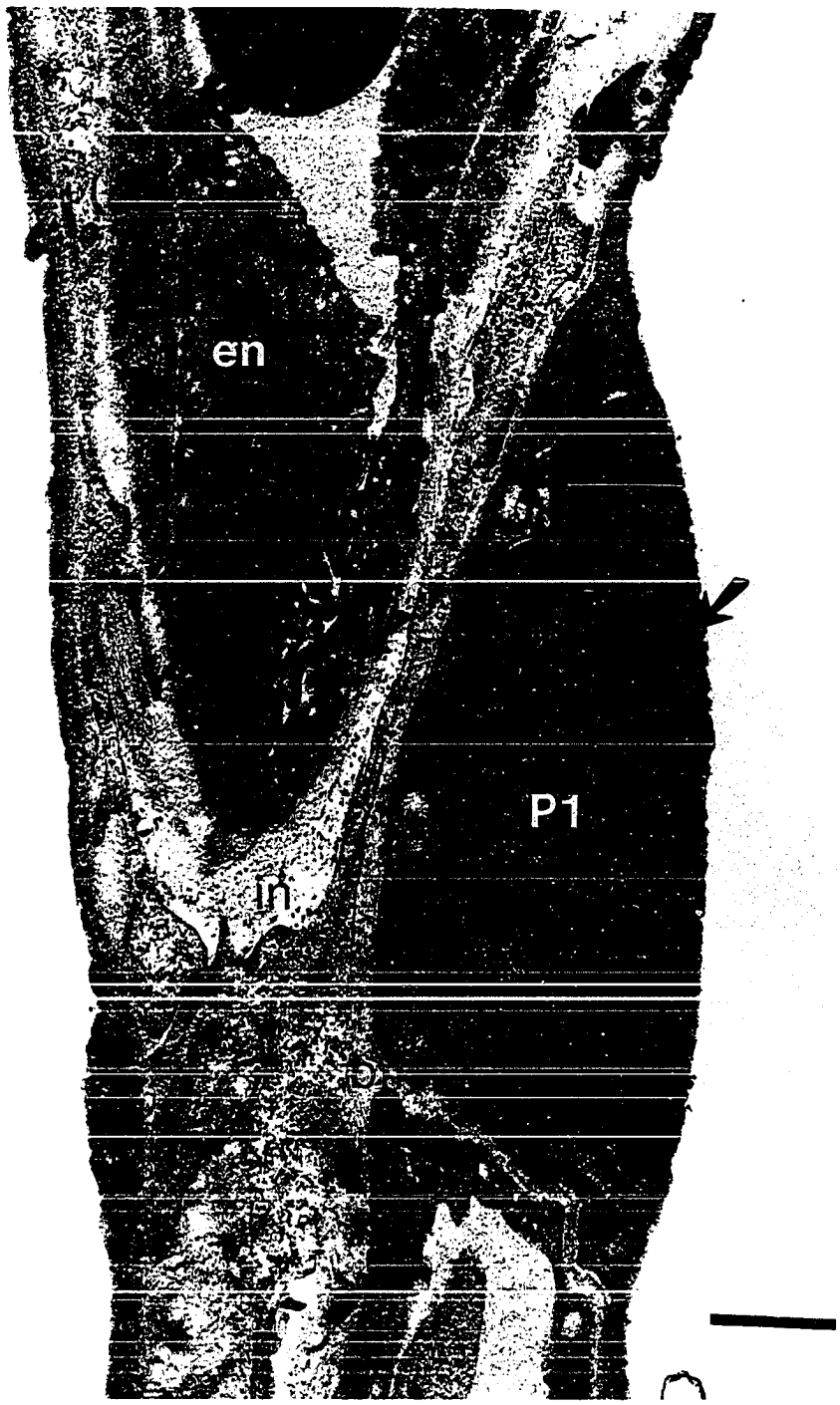
en
P1
in

Fig. 3: Alveolar wall from the same pig described in figure 2. A type 2 pneumocyte (P2) is shown. It has microvilli (mv) and lamellated surfactant granules (sg). Surfactant granule structure was not well preserved by in situ lung fixation. A fibroblast (fb) or pericyte is in the interstitium (in). Note pinocytotic vesicles in epithelial and endothelial cells (arrows). Uranyl acetate and lead citrate stain. Bar = 1 μm.

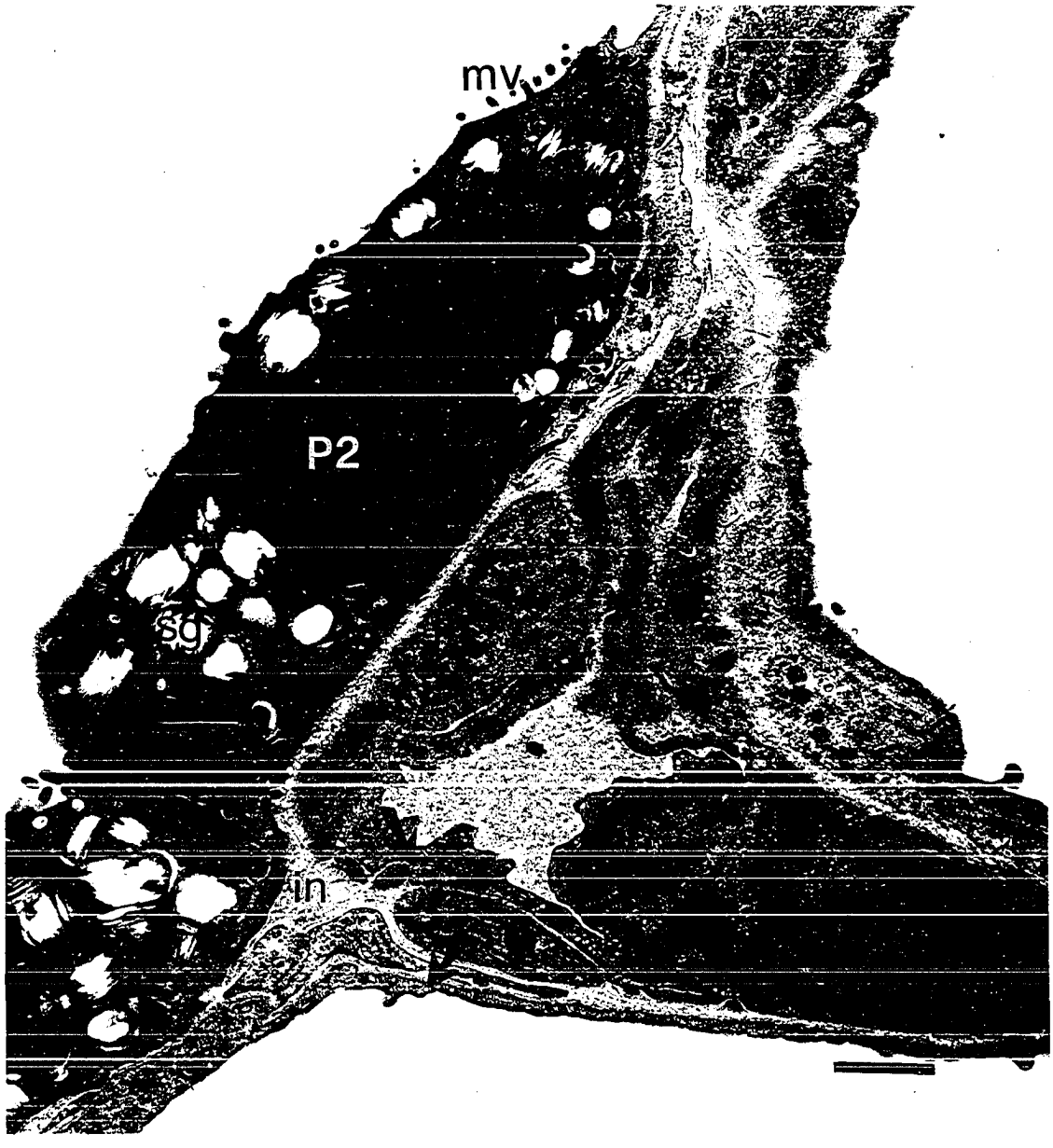
mv
P2
sg
in

Fig. 4: Predominantly neutrophilic response to soybean in swine lung at 1 day postinoculation. Neutrophils are within cotyledon cell protein bodies (pb) and along the periphery of protein bodies. The cell wall (cw) has a few macrophages (m) along its surface. Uranyl acetate and lead citrate stain. Bar = 1 μm.


pb

Fig. 5: A neutrophil has pseudopodia extending around protein body (pb) material. Plant material is in phagolysosomes (ph). Lesion was in lung of a conventional pig killed 1 day after receiving 20 ml of ethylene oxide-sterilized 15% soybean suspension. Uranyl acetate and lead citrate stain. Bar = 1 μm.

Fig. 6: Macrophages (m) and neutrophils (n) are phagocytizing soybean particles in the lung of a pig killed 3 days after receiving a single dose of soybean suspension. Soybean cotyledon cell protein body (pb) material is within phagolysosomes (ph) in many neutrophils. The cell wall (cw) is all that remains of one cotyledon cell. Uranyl acetate and lead citrate stain. Bar = 1 μm.

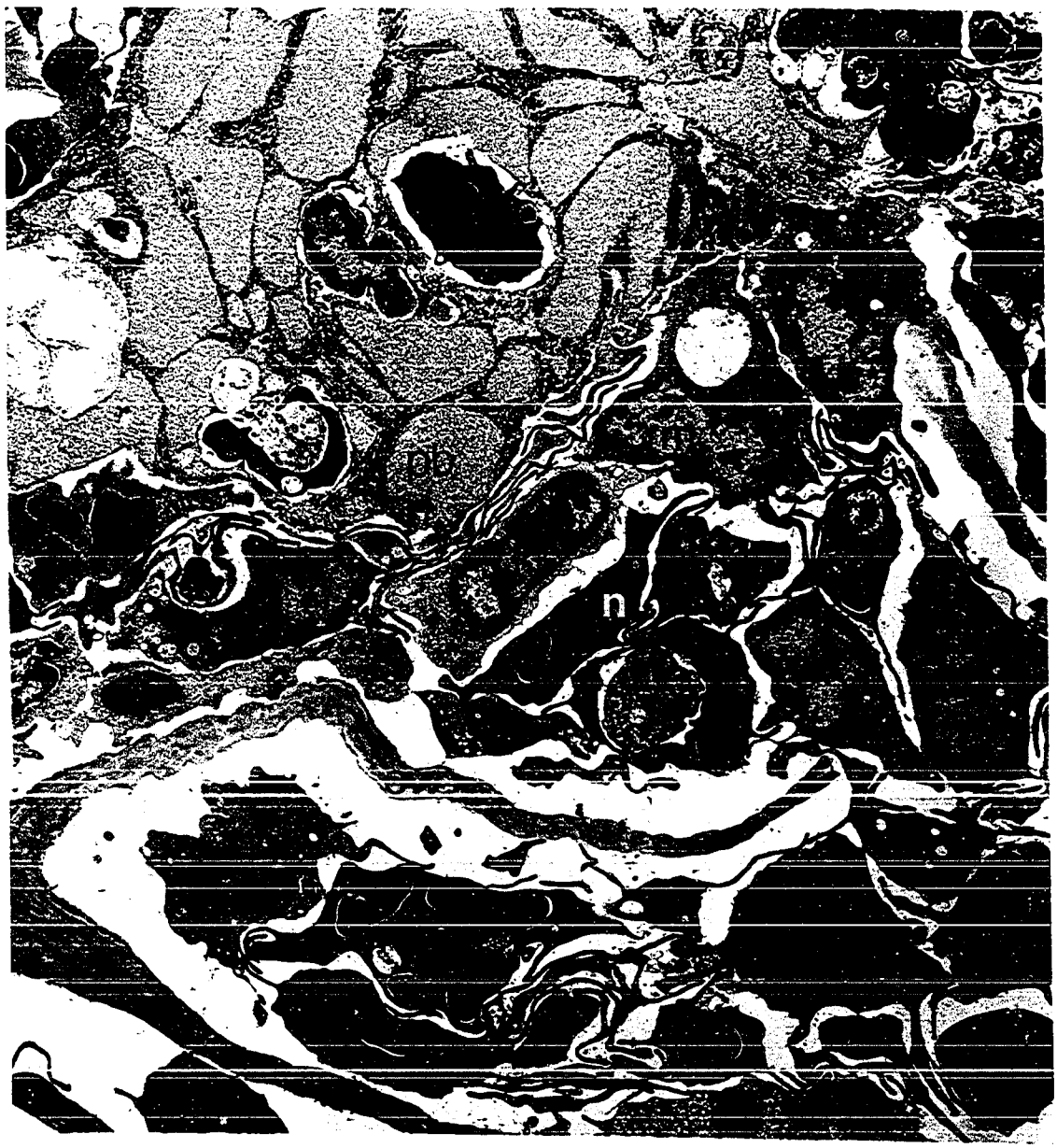
n

Fig. 7: Macrophages (m) are more abundant than neutrophils by 6 or 7 days after soybean inoculation. Cell wall (cw) is all that remains in cotyledon cells. Pseudopodia (ps) from macrophage extend through cotyledon cell wall. Uranyl acetate and lead citrate stain. Bar = 1 μm.

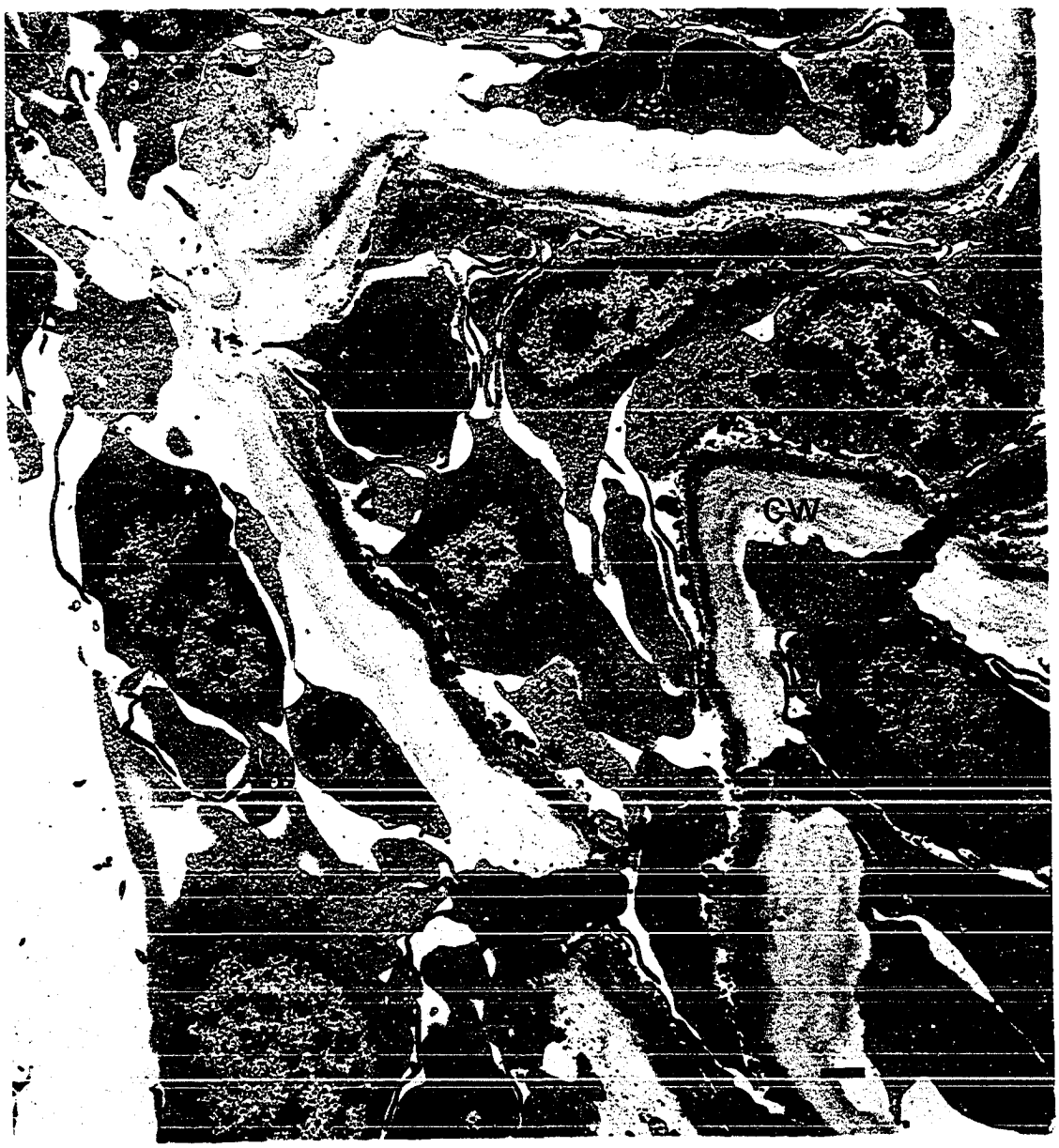
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Fig. 8: Several activated macrophages are loosely arranged around a soybean cotyledon fragment in the lungs of a pig killed 7 days postinoculation. Uranyl acetate and lead citrate stain. Bar = 1 μm.

Fig. 9: An aggregate of macrophages (m) is in an alveolus of a pig killed 7 days postinoculation. Note the interdigitation of macrophage processes, and plasma membrane fusion sites (fs), blurred zones along plasma membranes where cytoplasm of adjacent macrophages are merged to form multinucleated giant cells. Phagolysosomes (ph) are in some macrophages. Both type 1 (P1) and type 2 pneumocytes (P2) line the alveolus. Uranyl acetate and lead citrate stain. Bar = 1 μm.

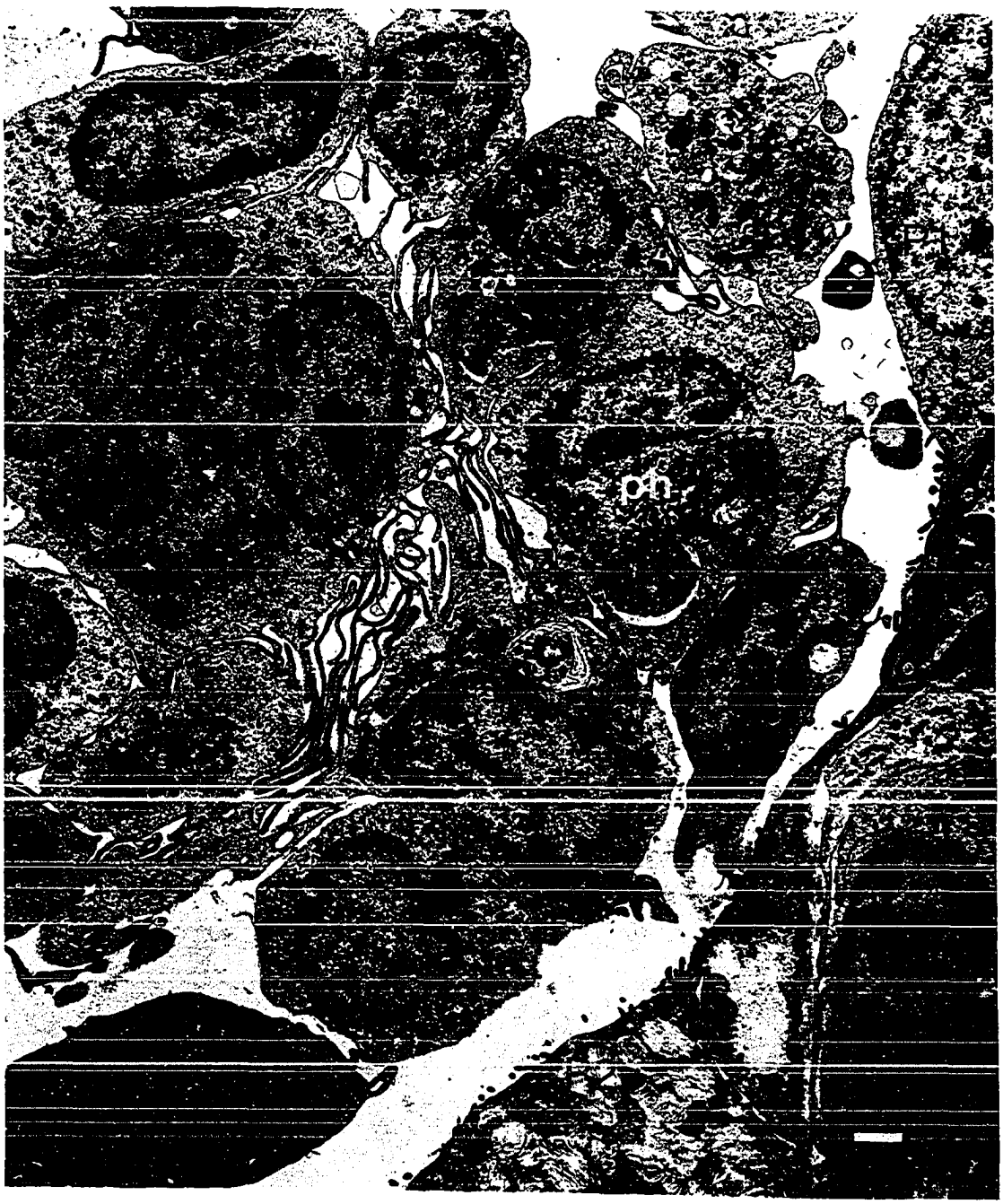
pn

Fig. 10: Macrophages are closely apposed to the epithelium of this alveolus (clear arrow). Note interdigitating macrophage processes with occasional fusion sites (solid arrow). Lung from a pig killed 7 days after receiving a single dose of soybean. Uranyl acetate and lead citrate stain. Bar = 1 μm.

Fig. 11: A multinucleated giant cell (MNGC) which does not contain plant material. This lesion was in a pig killed 7 days after receiving a single dose of soybean suspension. Uranyl acetate and lead citrate stain. Bar = 1 μm.

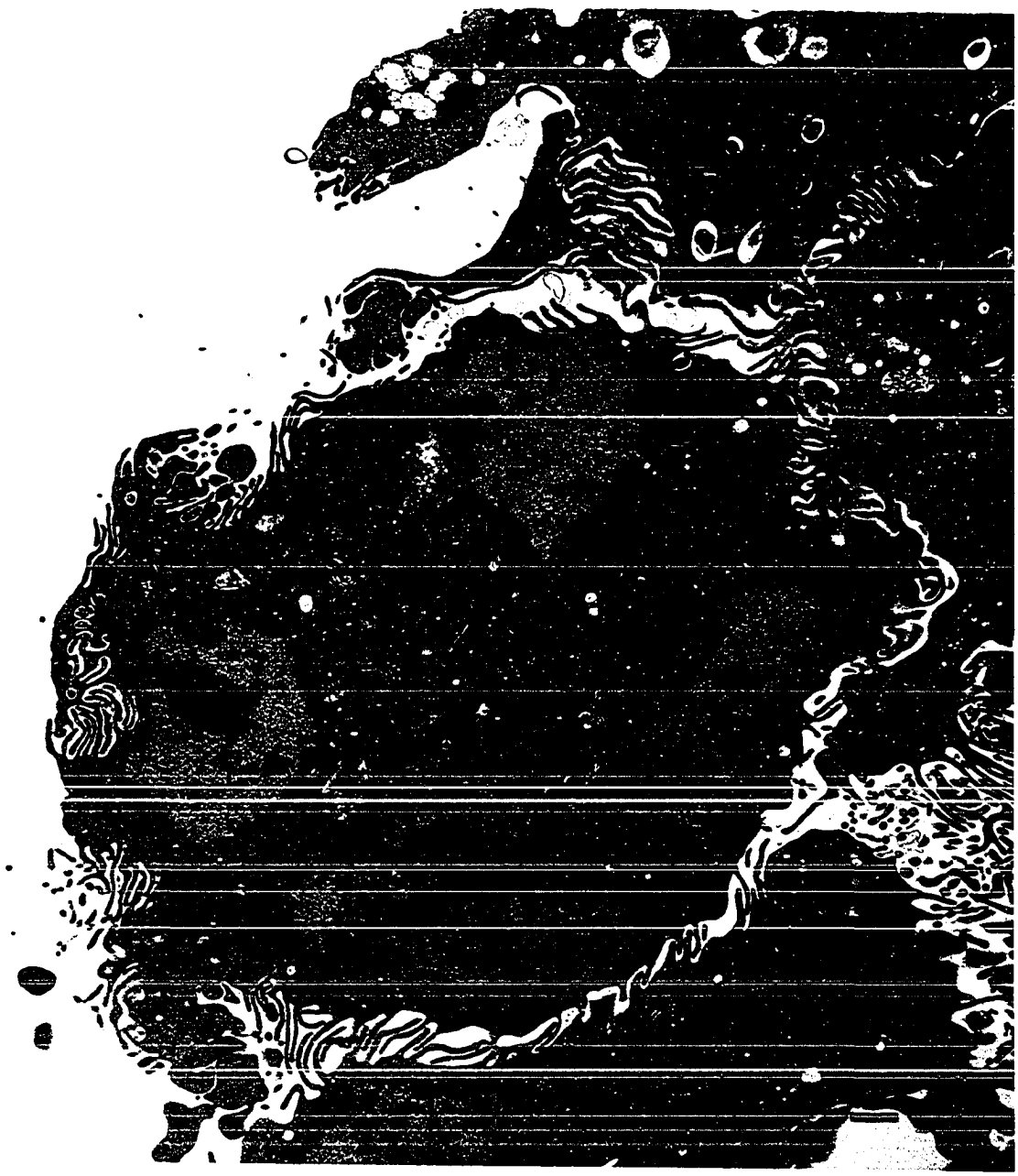

Fig. 12: This alveolus contains a multinucleated giant cell (MNGC) that is apposed to the alveolar basement membrane (bm). A pseudopod (ps) has penetrated the basement membrane at one point. The interstitium (in) is edematous. A fibroblast (fb) is adjacent to a capillary. The adjacent alveolus shows a type 2 pneumocyte (P2) that lines most of the alveolar surface. Uranyl acetate and lead citrate stain. Bar = 1 μm.

Fig. 13: A more chronic lesion in a pig, 14 days after receiving a single dose of soybean has disrupted basement membrane (bm), fibroblast (fb) proliferation and collagen (col) fibers which extend through the discontinuity in the basement membrane. A type 2 pneumocyte (P2) still lines a portion of the alveolus. The multinucleated giant cell (MNGC) has cytoplasmic processes which penetrate into the alveolar wall connective tissue. The epithelium of the adjacent alveolus is intact although type 2 cells are undergoing degenerative changes. Uranyl acetate and lead citrate stain. Bar = 1 μm.

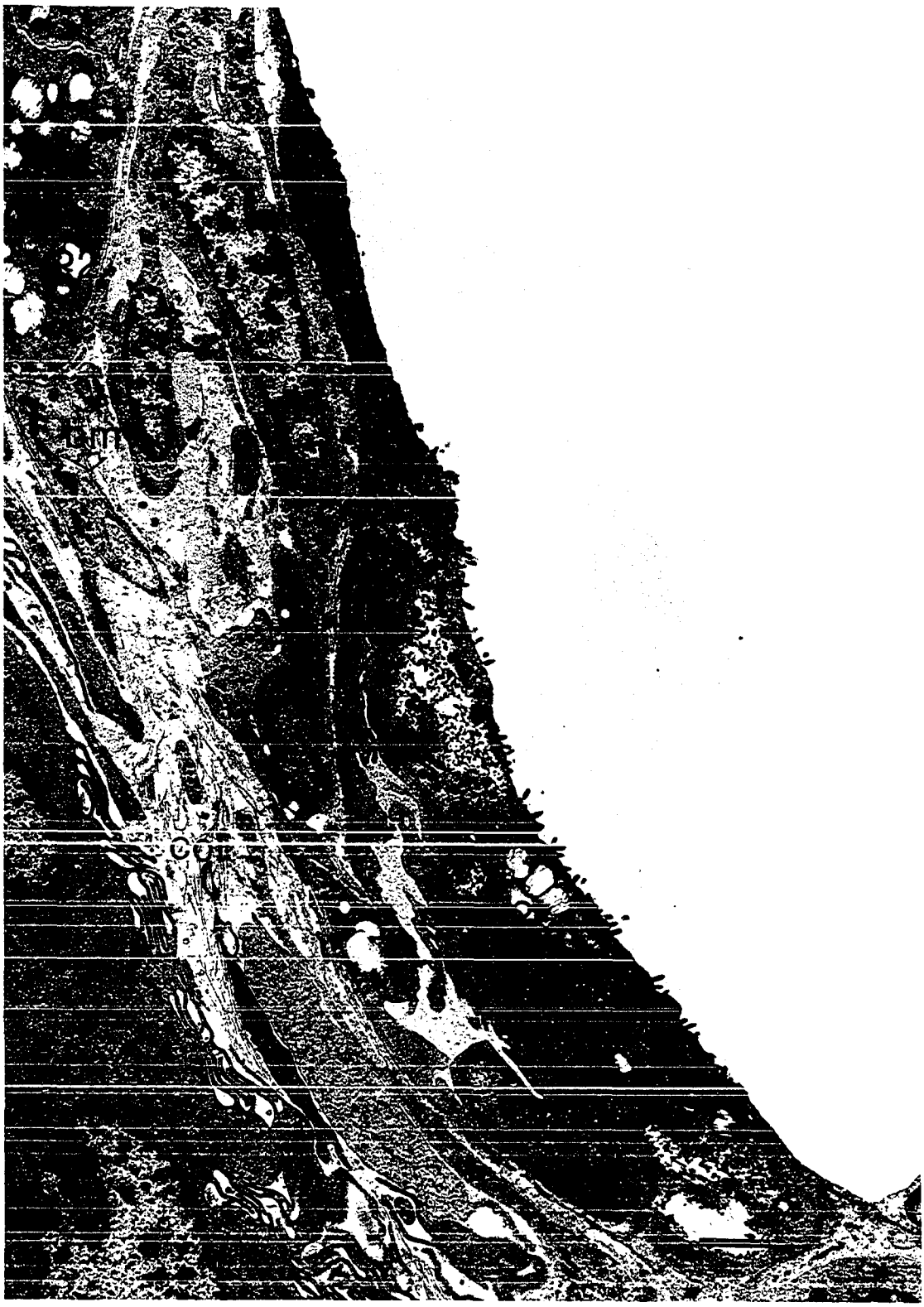

Fig. 14: Higher magnification of the lesion in figure 13. Dense regularly arranged collagen fibers (col) and a fibroblast (fb) are present deeper in the interstitium. The collagen fibers around the multinucleated giant cell (MNGC) are loosely arranged. Degenerative changes are in pneumocytes (P2) on both sides of the septum. Uranyl acetate and lead citrate stain. Bar = 1 μm.

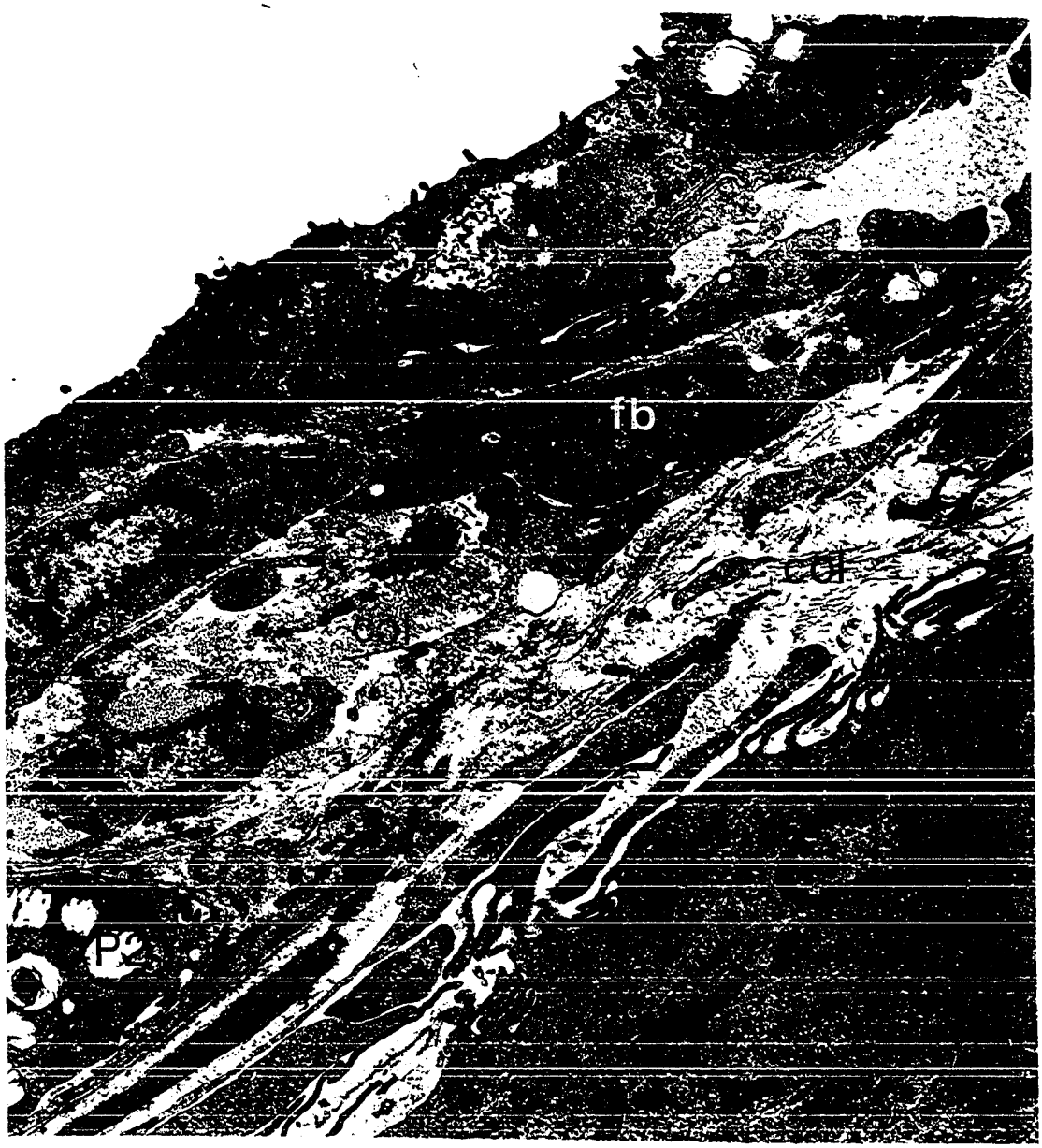
fb
col
PE

Fig. 15: Segment of an interstitial granuloma formed by 14 days postinoculation. Dense regularly arranged collagen (col) is abundant in the interstitium to include the zone immediately around the multinucleated giant cell (MNGC). Fibroblasts (fb) are increased in the interstitium. Arrow indicates a residual body in an epithelial cell. The epithelium of the adjacent cell is intact and shows type 2 pneumocyte (P2) hyperplasia. Uranyl acetate and lead citrate stain. Bar = 1 μm.

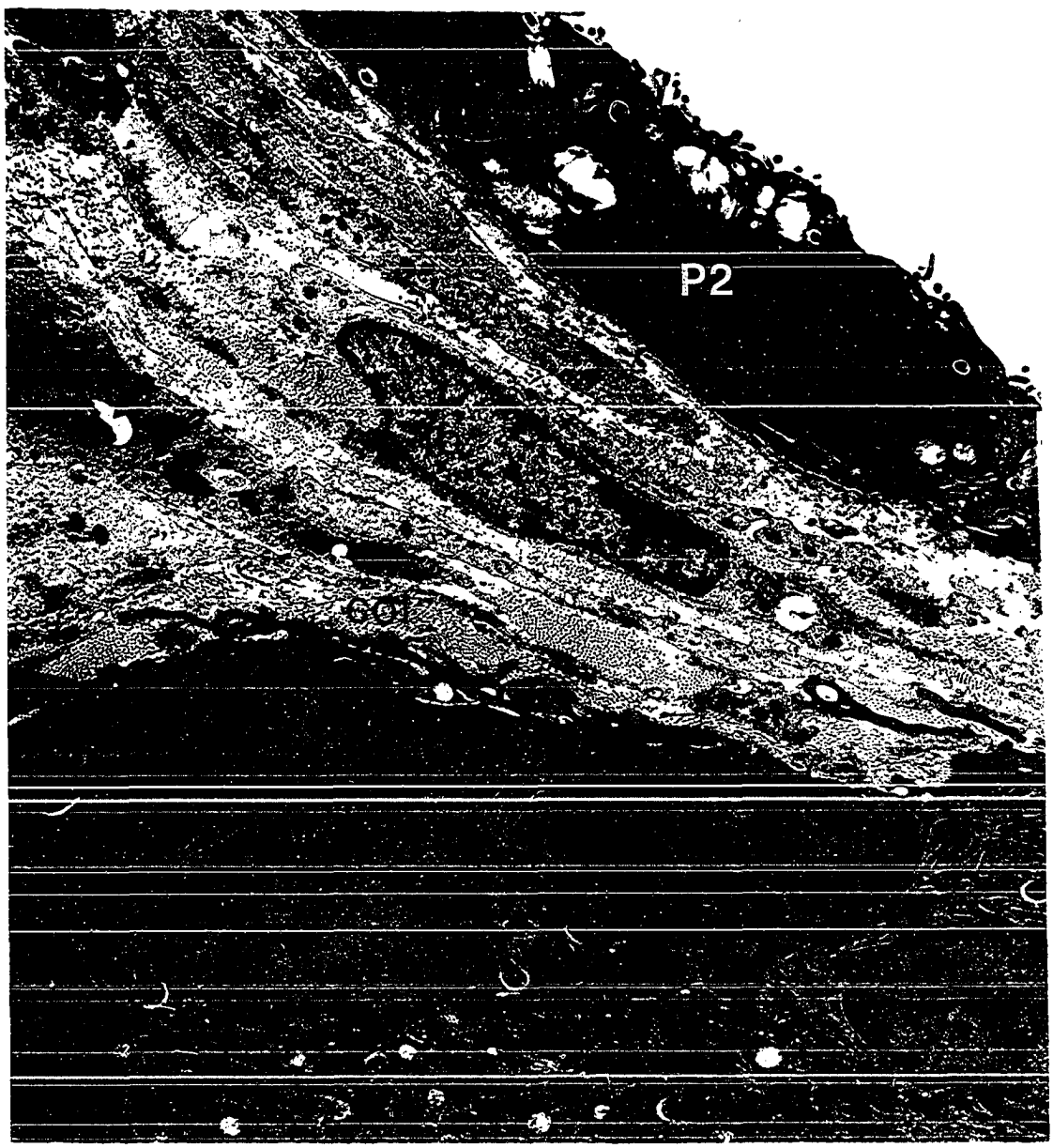
P2

Fig. 16: Type 2 pneumocyte (P2) hyperplasia and interstitial macrophage (m) infiltration is in lung of a pig killed 14 days postinoculation. Uranyl acetate and lead citrate strain. Bar = 1 μm.


P2
P2

GENERAL DISCUSSION AND CONCLUSIONS

This investigation revealed that the incidence of spontaneous plant-particle pneumonia in a single herd can be relatively high as evidenced by the finding of plant-particle pneumonia in 25 of 62 self-fed pigs from the same herd. Plant particles morphologically consistent with soybean were found in lung lesions of 7 of these 25 pigs. Six of 9 pigs from a 444-pig group studied retrospectively had naturally occurring plant-particle pneumonia lesions containing soybean. The presence of soybean in naturally occurring plant-particle pneumonia in swine has not been described previously (27,58,147).

Granulomatous inflammation was the predominant lung reaction in lungs of pigs with the natural disease. However, neutrophils were among multinucleated giant cells in consolidated lung sections. Bronchiolar occlusion by plant particles was also a characteristic feature of naturally occurring plant-particle pneumonia.

Previous reports described similar lesions of naturally occurring plant-particle pneumonia in swine (27,58,147). A high incidence of the disease in a single herd was also reported (27).

The finding of plant particles over 20 μm in alveoli and up to 260 μm in bronchioles suggests that aspiration of plant particles from the oral cavity may be the initial event leading to plant-particle pneumonia. Nasal filtration and nasal mucociliary transport remove most particles over 5 μm from inhaled air (131). However, it may also be possible that swine with severe atrophic rhinitis could inhale

particles over 5 μm into alveoli. Since plant particles were not found in lungs of floor-fed pigs but were found in lungs of self-fed pigs, feeding practice may be a disposing factor to spontaneous plant-particle pneumonia.

Experimental studies in conventional and respiratory disease free pigs revealed that sterile particles could cause coughing, dyspnea, gross and microscopic lung lesions. Therefore, "plant-particle pneumonia" is considered more appropriate terminology than "plant-particle associated pneumonia."

Gross bullous and hemorrhagic lung lesions may occur when air can be inspired but not expired as a result of bronchiolar obstruction and bronchospasm. Lung rupture is probably the fatal endpoint of bullous emphysema. Alveolar consolidation and atelectasis were the apparent causes of the yellow to gray mottling seen in lungs of pigs receiving multiple doses of soybean particles.

Three useful lung disease models resulted from histological and ultrastructural studies of lesions of experimental soybean-induced plant-particle pneumonia in swine: 1) Lungs of single-dosed pigs killed sequentially revealed the chronologic characteristics of plant-particle induced lesions. Suppurative bronchopneumonia was prominent 1 to 3 days postinoculation, thereafter granulomatous pneumonia was dominant. Interstitial granulomas, which may occur as early as 7 days, were prominent at 14 days postinoculation, while the quantity of inflammatory cells in alveoli had decreased. 2) Lesions in multiple-dosed pigs killed at 1,

3, or 6 days after receiving 3 doses of soybean particles given over a 10 day period were simultaneously suppurative and granulomatous. Therefore, this model is considered representative of naturally occurring plant-particle pneumonia. 3) Ultrastructural study of the lung inflammatory response to soybean particles showed that multinucleated giant cells with or without recognizable plant particles could become entrapped in alveolar interstitium after alveolar epithelium and stroma were damaged. Enzymes secreted by multinucleated giant cells and macrophages may account for alveolar damage (13,143,144). Consequently, the functional capacity of an alveolus may be lost when a granuloma is completely encircled by connective tissues. Since particles that were usually 20 to 100 μm were found in interstitial granulomas of pigs with naturally occurring plant-particle pneumonia, this mechanism of interstitial granuloma formation could explain how particles too large to penetrate alveolar epithelium become translocated into the alveolar interstitium.

Naturally occurring and experimental plant-particle pneumonia lesions observed in these studies resemble lesions described for lentil pulse pneumonia in humans (51,64,137) and experimental animals (64). Similar lesions occurred in bucket-fed calves that aspirated milk replacer containing linseed particles. Acute bronchopneumonia followed by granulomatous pneumonia was also reported for experimental lentil pulse pneumonia (64) and experimental pine pollen granuloma (139) in laboratory animals.

In conclusion, the high incidence of spontaneous plant-particle pneumonia found in this study suggests that the disease may be even

more common than would be suggested by the few existing reports (27, 58,147). Soybean may be significant in the natural disease, since it was found in spontaneous lesions and can cause disease without bacterial interaction. The method of feeding may predispose to multiple lung exposures, which are believed to be responsible for lesion characteristics in pigs with naturally occurring plant-particle pneumonia. Further studies on natural plant-particle pneumonia are indicated to characterize epizootiologic factors, clinical signs, gross lesions, and effects on swine performance. The possible interaction of plant particles with respiratory pathogens also requires further study.

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ACKNOWLEDGEMENTS

The timely guidance and complete support given by Dr. H. J. Barnes throughout my graduate degree research and preparation of this dissertation are greatly appreciated. His keen insights, patience, kindness and encouragement were invaluable contributions to the enriching experiences which I had as a graduate student.

I wish to thank Dr. J. P. Kluge for his early guidance of my coursework and for his assistance as a co-major professor throughout my graduate training.

I am also grateful to the other members of my graduate committee, Drs. W. P. Switzer, R. F. Ross and T. L. Carson for their time and advice given in support of my graduate program, and for their constructive suggestions regarding this dissertation.

My gratitude is extended to Drs. P. M. Gough, L. B. Hall, K. B. Platt, R. F. Ross, and G. N. Woode for providing conventional pigs used in experiments reported in this manuscript.

Collectively, I wish to thank the staffs of the Veterinary Medical Research Institute, Veterinary Pathology Department and Biomedical Communications Center for their enthusiastic assistance given in support of my research and academic work.

Special thanks are expressed to Mr. A. S. Helland for coordinating animal facilities; Mr. R. O. Ross for histotechnology assistance; Ms. J. A. Olsen for electron microscopy and photographic printing assistance; Ms. B. J. Zimmermann for microbiology assistance;

Ms. T. F. Young for serology assistance; and Mr. D. Stambaugh and Mr. D. W. Biechler for audiovisual support.

My appreciation is also extended to Mrs. K. S. Pierce for histotechnology and Mrs. J. A. Fagerland for electron microscopy assistance which supported my coursework and research needs.

My gratitude is also expressed to Mr. M. O'Donnell, Ms. R. Brady, Ms. K. McClintock, Ms. R. Scholey, and Mr. J. Delano for assisting with various animal experimentation tasks.

The patience and expertise of Mrs. Joan Lang in typing the master copy of this manuscript is deeply appreciated.

This research was supported by USDA Farm Bill (PL 95-113) Formula Funds. I am very grateful to the U.S. Army Veterinary Corps and U.S. Army Medical Department for providing this educational opportunity, tuition and personal financial support, and partial funding for preparation of this dissertation.

The patience and encouragement of my wife, Ollie, and children, Maria and Stanley, during my graduate program are deeply appreciated. Their independence and enthusiastic cooperation greatly facilitated my graduate studies. I also thank my wife for typing draft copies of this manuscript.